The Chemoenzymatic Preparation of Optically Active Molecules.

By

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To Mum, Dad and Auntie Jean.

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DECLARATION

The work described in this thesis is the original of the author, except when acknowledgement has been made to results and ideas previously mentioned. The work was carried out at three institutions:

(1) The Department of Chemistry, The University of Warwick (October 1986 to April 1988 and October 1989 to October 1990).

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(3) The School of Pharmacy, The University of Wisconsin-Madison (August 1983 to August 1989).

The work detailed in Chapters Three, Four, Five and the related experimental details in Chapter Seven was carried out at The University of Wisconsin-Madison, under the supervision of Professor C. J. Sih. At the time the author was a Graduate Exchange Fellow, sponsored by The Universities of Wisconsin-Madison and Warwick. The work described in this thesis has not been previously submitted for a degree at any institution.

SUMMARY

This thesis describes the use of enzymes in organic synthesis and their application to produce optically active compounds, either by enantiospecific or enantioselective reactions.

Chapter two describes the enantioselective synthesis of a β-blocker, Betaxalol. Initial experiments to enantioselectively epoxidise a pro-chiral alkene precursor failed. However, methane monooxygenase (*ex Methylococcus capsulatus* (Bath)) epoxidised allyl bromide and allyl chloride, the allyl chloride produced was racemic. Microbiological reduction of an α-chloroketone gave optically enriched α-chloroketone. The butanoate of the α-chloroketone was enzymatically resolved. However, other undesired hydrolysis reactions were occurring. Accordingly, a compound more closely related to Betaxalol was chosen for further investigation. A screen of ten enzymes revealed that racemic 3-[4-[2-(cyclopropylmethoxy)phenyl]-1-chloro-2-acetoxypropane was enantioselectively hydrolysed by the lipase from *Pseudomonas fluorescens* (E=14). Substrate modification gave the corresponding butyrate as the optimised structure (E=35). *Pseudomonas fluorescens* catalysed esterification of the corresponding chlorohydrin, was attempted. The best acyl donor was vinyl acetate (E>100). Both enantiomers of 3-[4-[2-(cyclopropylmethoxy)phenyl]-1-chloro-2-acetoxypropane (>98%ee) were produced and converted in two steps into optically pure β-blocker. Reduction of the corresponding α-chloroketone was less successful.

Chapter three describes the synthesis of a protected form of 4-amino-2-oxobutanoic acid. Twenty yeasts were then screened for enantiospecific reduction of the ketone. The best yeast was *Saccharomyces carlsbergensis* ATCC 2345, which produced (2S)-methyl-4-(benzyloxycarbonyloxy)-4-amino-2-hydroxybutanoate (88%ee). The stereochemistry was determined by subsequent chemical conversion to (3S)-3-hydroxy-2-pyrrolidine.

Chapter four describes the yeast reduction of 4-(phenylthio)butan-2-one and related ketones. Both (2R) and (2S), 4-(phenylthio)butan-2-ol (>98%ee) were produced by screening 68 yeast strains. Attempts to scale-up the yeast reduction are described.

Chapter five describes the stereospecific hydrolysis of *cis*-3,6-diacetoxycyclohexene. 23 hydrolytic enzymes were screened. The Lipase P-30 (*Pseudomonas sp.*) produced (+)-(3S,6R)-3-hydroxy-6-acetoxycyclohexene in 64% yield, 79%ee. The absolute stereochemistry was established by subsequent chemical conversion to (+)-(1S,6R)-7-oxabicyclo[4.3.0]non-2-en-8-one.

Chapter six describes approaches to optically pure aziridine-2-carboxylates. Direct resolution of ethyl 3-phenyl-1H-aziridine-2-carboxylate was attempted. The *cis* racemic pair was hydrolysed. The *trans* racemic pair could not be hydrolysed (9 enzymes screened). Resolution of ethyl 3-methyl-3-azido-2-acetoxybutanoate was more successfully. From a screen of 9 enzymes the lipase from *Candida cylindracea* was selected (E>100). Racemic ethyl 3-methyl-3-azido-2-hydroxybutanoate was converted to ethyl 3,3,dimethyl-1H-aziridine-2-carboxylate.

ABBREVIATIONS

n.m.r.	Nuclear magnetic resonance spectroscopy.
ppm	Parts per million.
TMS	Tetramethylsilane.
J	Coupling constant.
t.l.c.	Thin-layer chromatography.
g.l.c.	Gas-liquid chromatography.
PLC	Preparative layer chromatography.
FC	Flash chromatography.
HRMS	High resolution mass spectrometry.
ee	Enantiomeric excess.
$[\alpha]$	Specific rotation at 589 nm.
c	Concentration (g/100ml).
de	Diastereomeric excess.
E	E value.
m.p.	Melting point.
b.p.	Boiling point.
min	Minutes.
hr	Hours.
THF	Tetrahydrofuran.
DMSO	Dimethylsulphoxide.
DMAP	4-Dimethylaminopyridine.
MPTA	2-Methoxy-2-Trifluoromethylphenylacetyl.
Ac	Acetate.
Et	Ethyl.
Me	Methyl.
Ar	Aryl.
NAD	Nicotinamide adenine dinucleotide.
NADH	Nicotinamide adenine dinucleotide, reduced form

CHAPTER ONE

1.1 INTRODUCTION

Over the last two decades chemists have, like a prodigal son, returned to using enzymes in organic synthesis.

The catalyst for this return has been an increased awareness in the scientific community and the public in general that chiral drugs should be sold as optically pure compounds.[1]

About 50% of the 1,800 drugs currently marketed are racemates.[1] Frequently the biological activity is attributable to only one enantiomer. If the other enantiomer is not metabolised and excreted efficiently, then it may cause detrimental side effects.

Unfortunately, side effects have already been observed. The drugs Thalidomide and Benoxaprofen provide classic examples.[2]

So, convinced of the need for enantiomerically pure compounds (E.P.C.) chemists have set about developing methods to enable them to produce optically pure compounds.[3]

All current strategies rely on an already present chiral centre to act as a template to control/direct asymmetric induction.

Present methods include :

(1) Synthesis starting from naturally occurring chiral molecules e.g. amino acids and carbohydrates.

(2) Resolution of enantiomers.

(3) Asymmetric synthesis.

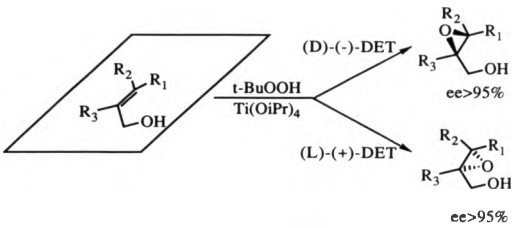
Syntheses starting from compounds in " The Chiral Pool " are often expensive and lengthy. However a plethora of excellent examples are available.[4] One of the advantages of using a sugar[5] or amino acid[6] is that the absolute configuration of the starting material is known. Therefore by closely studying the synthetic sequence the configuration of the desired product is automatically established.

The scope of this thesis lies within the realms of the two other approaches to E.P.C. production, namely resolution and asymmetric synthesis.

Chemical resolution involves the formation of diastereomeric complexes which are physically separable. These complexes can be chemically bonded such as ephedrine salts,[7] or transient species such as during passage of a chiral analyte through a chromatography column possessing a chiral stationary phase.[8] Classical resolution is still the mainstay of chiral synthesis. However the method can be time consuming and laborious. Chiral columns are not readily amenable to large scale use.

Asymmetric synthesis involves the construction of a chiral template, or catalyst to direct asymmetric induction. Two superb examples have emerged (Schemes 1.1 and 1.2).

(1) The Sharpless asymmetric epoxidation catalyst[9,10]

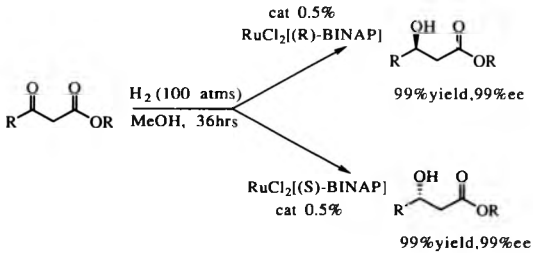


Scheme 1.1

DET = Diethyl tartrate

R_1 must be small

(2) Noyori's asymmetric hydrogenation catalyst[11]



Scheme 1.2

Instead of designing and constructing an asymmetric catalyst, one can turn one's attention to ready made catalysts, namely enzymes.

The study and use of enzymes as chiral catalysts gives the chemist a choice of how best to perform a given transformation. Obviously the more methods available the more efficient the overall transformation will be.

Enzymes are biopolymers. Basically they consist of one or more linear chain(s) of amino acid residues. This primary structure is then locally folded to give discrete secondary structures (α-helixes, β-sheets, etc). The various secondary structures interact to produce the folded tertiary structure. If there is more than one tertiary structures these combine to give the quaternary structure, this is known as the *apo*enzyme. Several enzymes require additional cofactors, prosthetic groups or cosubstrates essential for activity. The arrangement of *apo*enzyme and cofactor is termed the *holo*enzyme.[12]

Most enzymes function best in a closed environment, the cell, where factors such as pH and temperature are strictly regulated. They can either be membrane bound or soluble.

The word enzyme means "in yeast". The term was coined by Eduard Buchner in 1897, who when trying to preserve yeast extract in a sugar solution, found, to his astonishment, that a gas was evolved.[13] This was contrary to the dogma of the day which stated that the whole intact microbe was required for

fermentation.[13]

"Biotransformations" is the term given to the use of enzymes in organic synthesis. Although no definitive definition has been accepted the following quotation aptly describes biotransformations:[14]
"(The) selective enzymatic conversion of natural or chemically synthesised substrates into defined products on a preparative scale using whole cells or isolated enzyme systems".

One of the first Biotransformations was carried out by Le Bel in 1881 who resolved racemic propane-1,2-diol using *Bacterium termo*.[15] The microorganism stereoselectively metabolised one enantiomer, the untouched enantiomer was recovered and chemically converted into optically active propene oxide.

To carry out a biotransformation one must first select an enzyme. There are at least 2,477 enzymes known and over 300 are commercially available in various states of purity.[16]

The Enzyme Commission in 1955 conveniently gave each enzyme a unique E.C. number (E.C. w.x.y.z.). All enzymes belong to one of six classes (w = 1 to 6) dependent on the type of reaction catalysed:[17]
(1) Oxidoreductases
(2) Transferases
(3) Hydrolases
(4) Lyases (elimination)
(5) Isomerases

(6) Ligases (also known as synthetases)

Biotransformations have mainly been carried out using enzymes from classes (1) and (3)[18] and in what follows, attention will be directed towards the oxidoreductases and hydrolases. There are however many examples from classes (2) and (6).[19] The use of ligases will surely increase as the ability to form carbon-carbon bonds is a central theme in organic chemistry.

Enzymes have several advantages over conventional chemical reagents in that they are:
(a) Selective, often exhibiting regio- and stereoselectivity.
(b) They can operate at or near neutral pH under mild conditions of temperature and pressure. This is especially useful when dealing with sensitive compounds.

Many reviews on biotransformations have been published.[14,15,17,18,20,21,22,23,24,25,26] Each chapter in this thesis has a specific introduction. However, at this point a brief synopsis of some previous applications of oxidoreductases and hydrolases in organic synthesis, will be given.

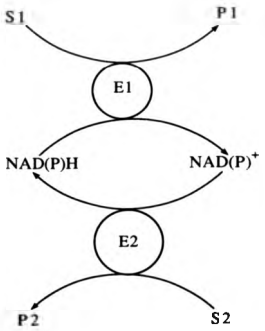
1.2 OXIDOREDUCTASES

This class of enzymes can be further subdivided into:
Oxygenases
Dehydrogenases
Oxidoreductases require a hydride donor or acceptor. This is accomplished by the use of cofactors such as flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN) or more

frequently a cosubstrate such as nicotinamide adenine dinucleotide (NADH). If the biotransformation is carried out using an isolated enzyme then a molar equivalent of cofactor/substrate will also have to be added, this is prohibitively expensive, unless the reaction is done on an analytical scale.[27]

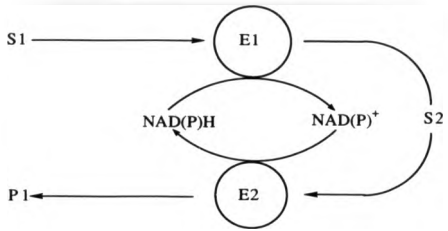
The problem can be surmounted in one of two ways:
(1) The cofactor/cosubstrate can be recycled. This area has received a lot of attention. NAD^+ can successfully be recycled to NADH when used as a cosubstrate for dehydrogenases in the reductive direction (Schemes 1.3, 1.4 and 1.5).[27]

(A) Recycling applying a second enzyme and second substrate



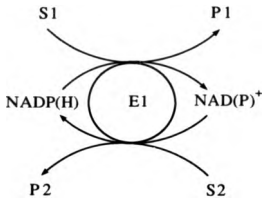
Scheme 1.3

(B) Substrate-coupled recycling



Scheme 1.4

(C) Coupled-substrate recycling



Scheme 1.5

E = Enzyme
S = Substrate
P = Product

However recycling of NADH to NAD^+ still requires some attention. The problem is complicated in this case because the

position of equilibrium is unfavourable and severe product inhibition can occur.[27]

(2) The biotransformation can be carried out using the whole cell. Here the cofactor/cosubstrate is automatically recycled by the cellular enzyme system. Each solution has it own advantages and disadvantages which can be summarised (Table 1.1)

Table 1.1 The advantages and disadvantages between using whole cells and isolated enzymes.

	Advantages	Disadvantages
Enzyme	Trivial work-up Convenient Specific Immobilisation easy Active site more accessible	Expensive Instability problems Cofactor recycling neccesary
Whole cell	Cofactors automatically recycled Cheap Certain enzymes inducible	Low substrate concentrations obligatory Microbiological skills required Polar substrates unable to enter cell Tedious and difficult work up Biosynthetic pathways may lower yield Competing enzymes interfere

Even though the problem of some cofactor/cosubstrate recycling has been worked out, most biotransformations involving oxidoreductases have been carried out employing whole cells.[18]

1.2.1 OXYGENASES

Oxygenases can be divided into two separate groups. The dioxygenases incorporate both atoms of molecular dioxygen into

the substrate (Scheme 1.6).[28,29]

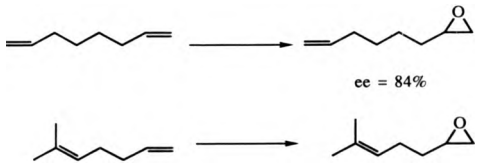
S = substrate

Scheme 1.6

The monooxygenases, as their name suggests, incorporate only one atom of molecular dioxygen into the substrate, whilst the other is reduced to water (Scheme 1.7).[30]

Scheme 1.7

The reducing power is supplied by a cosubstrate, normally NADH or NADPH. Examples of oxygenation reactions include:
(1) The regio- and stereospecific epoxidation of terminal double bonds by the ω-hydroxylation system from *Pseudomonas oleovorans* (Scheme 1.8).[31,32]



Scheme 1.8

(2) The production of chiral sulphoxides. An example worthy of special mention is the microorganism *Corynebacterium equi*

IFO 3730 (this bacterium has been reclassified[34] as *Rhodococcus equi* IFO 3730) which can oxidise several alkyl-aryl and allyl-aryl sulphides with phenomenal stereospecificity (Table 1.2).[33]

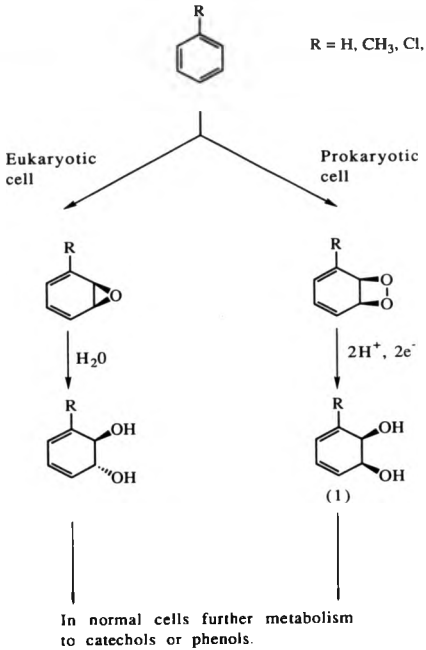
Table 1.2 Enantiospecific sulphoxide production using the microorganism *Corynebacterium equi* IFO 3730.

R_1	R_2	%yield	%ee
Ph	$n\text{-}C_{10}H_{21}$	25	99
Ph	$n\text{-}C_4H_9$	29	100
Ph	$CH_2{=}CHCH_2$	38	100
$Me\text{-}C_6H_4$	$CH_2{=}CHCH_2$	67	92
$Me\text{-}C_6H_4$	$n\text{-}C_{10}H_{21}$	55	91
Ph	CH_3	100	75

(3) The hydroxylation of unactivated alkanes to alcohols. Examples are seen in the steroid field where by judicious choice of microorganism almost every carbon atom can be hydroxylated with specified stereochemistry.[35]

(4) Baeyer-Villiger oxidations.[36]

(5) Hydroxylations of aromatic compounds. Here a remarkable difference in stereochemistry is observed depending on whether a prokaryotic or eukaryotic dioxygenase is utilised (Scheme 1.9).[37]



Scheme 1.9

Further oxidation to a catechol is prevented if a mutant strain is used. A mutant from the prokaryotic microorganism *Pseudomonas putida*, for example, has been used successfully to produce *cis*-1,2-dihydroxy-3,5-cyclohexadiene [(1) in Scheme

1.9, R=H] (the so-called benzene *cis* diol). This compound has then been incorporated into several synthetic sequences.[38,39]

(6) Oxygenases can catalyse the oxidative cleavage of O- and N-methyl groups (Scheme 1.10).[40]

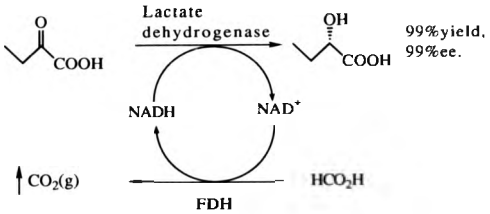
$R_2NMe \longrightarrow R_2NH + MeOH$

$ROMe \longrightarrow ROH + MeOH$

Scheme 1.10

1.2.2 DEHYDROGENASES

This sub-class of enzymes has been widely used for the production of chirons. The most common application is the stereospecific reduction of ketones to secondary alcohols.[41,42] Alcohol dehydrogenases require a hydride donor in the hydrogenation direction, this is usually supplied by the cosubstrate NADH or NADPH. As usual the cosubstrate must be recycled. Perhaps the best method is the one that makes use of the formate/formate dehydrogenase (FDH) system (Scheme 1.11), since the end product (CO_2) bubbles out of solution and so product inhibition is minimised.[43]



Scheme 1.11

Because of cofactor problems, as with other redox enzymes, the majority of biotransformations are mediated by whole cells. The most commonly used microorganism is *Saccharomyces cerevisiae* (baker's yeast). An extensive review has recently been published by Servi which covers work in the area over the last fifteen years.[44] Reactions mediated by baker's yeast are remarkably simple to operate; requiring tap water, readily obtainable yeast and sometimes sugar.

In the 1950's V. Prelog carried out a stereochemical study of the reduction of mainly bicyclic ketones mediated by *Curvularia falcata* and observed hydride addition to the pro R (*re*-face) to give the (S)-alcohol (Scheme 1.12).[45]

S = Small group
L = Large group

Scheme 1.12

This became known as "Prelog's Rule"[46] and it can be loosely applied to yeast alcohol dehydrogenase (YADH), a tetrameric enzyme isolated from baker's yeast.[47] YADH can only reduce aldehydes or methyl ketones.[48] The archetypal substrates for yeast reductions are β-ketoesters (Scheme 1.13).[44]

Prelog's Rule observed (S)-(L)

Scheme 1.13

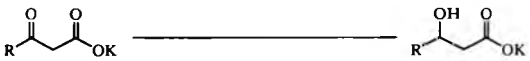
However when the R group is larger than methyl the "Anti-Prelog" compound is formed (Scheme 1.14).[49]

Prelog's law not observed (R)-(D)

Scheme 1.14

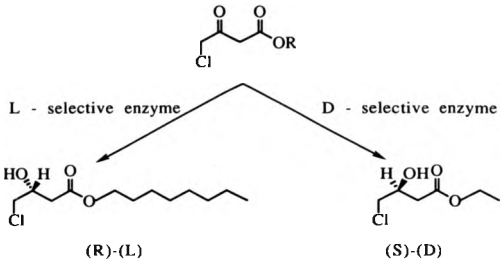
This has also been observed when a series of the free β-keto acids (as their corresponding potassium salts) were reduced (Table 1.3).[49]

Table 1.3 Effect of chain length on the baker's yeast mediated reduction of a series of β-keto esters.



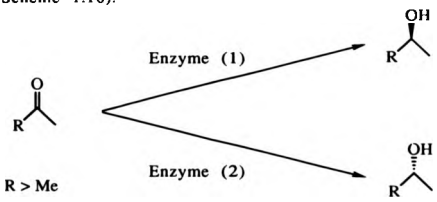
R	R/S	L/D	%ee	% yield
Me	S	L	>96	34
n-$C_{15}H_{31}$	R	D	98	40
n-C_4H_9	R	D	-	26
n-C_3H_7	R	D	-	16
CH_2=$CHCH_2CH_2$	R	D	>98	35

In fact three dehydrogenases have been isolated, purified to homogeneity and partially characterised. In the old D/L nomenclature two enzymes are D selective and the other is L selective (Scheme 1.15).[50]



Scheme 1.15

The presence of more than one dehydrogenase leads to a potential problem. If, for example, two enzymes of opposite stereochemistry reduce a given prochiral ketone then the overall reduction will proceed with incomplete stereospecificity (Scheme 1.16).[51]



Scheme 1.16

For enzymes obeying Michaelis-Menton conditions, the ratio of rates of the two competing enzymes is given by:

$$\frac{V_1}{V_2} = \frac{(k_{cat}/K_m)\ [E_1]}{(k_{cat}/K_m)\ [E_2]}$$

Where:
V = Overall rate
k_{cat} = Turnover number
K_m = Michaelis constant
[E] = Enzyme concentration

One can often circumvent the problem by the slow addition of substrate so that the effective concentration of substrate is always less than the saturation concentration of the active sites of the enzymes ([S] < K_m). If this condition is observed then the enzyme with the greater (k_{cat}/K_m) will reduce the substrate (Scheme 1.17).[52]

If

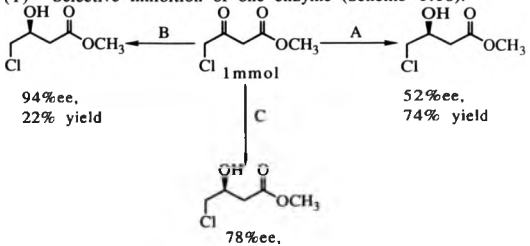
$$\left(\frac{k_{cat}}{K_m}\right)_1 > \left(\frac{k_{cat}}{K_m}\right)_2$$

therefore $V_1 > V_2$

Scheme 1.17

If the desired reduction is still unobtainable with baker's yeast then several other strategies are available:

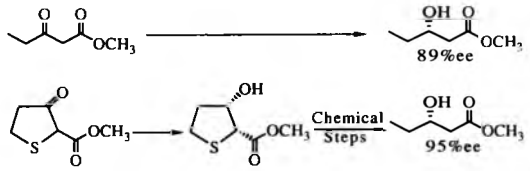
(1) Selective inhibition of one enzyme (Scheme 1.18).[53]



A = baker's yeast
B = baker's yeast plus 0.5 mmol methyl vinyl ketone
C = baker's yeast plus 1 mmol allyl alcohol

Scheme 1.18

(2) Substrate modification, especially if the substrate is temporarily cyclised (Scheme 1.19).[54]



Scheme 1.19

(3) Cell immobilisation.[55]

(4) Alteration of the growing media especially if the desired

enzyme is inducible.[56]

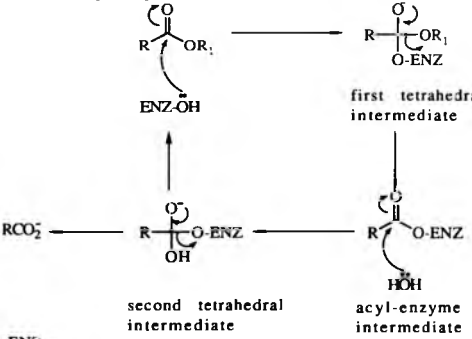
(5) Screening of other microorganisms.[25] This is a very useful method and can produce beneficial results in a relatively short period of time. However the method is very empirical - the more microorganisms screened the more likely the desired result will be obtained.

1.3 HYDRO..ASES

Hydrolases, like dehydrogenases, have been widely used. Over one hundred hydrolases are commercially available.[57] The methods by which they are used are uncomplicated. Many of the enzymes are cheap and easily obtainable. However by far the biggest advantage is that hydrolases do not require cofactors and so isolated enzymes can be used.

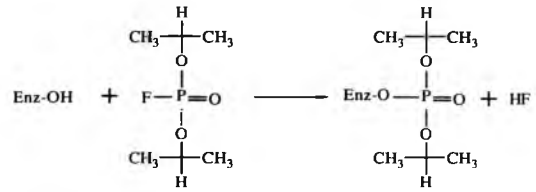
The enzymes most commonly applied are α-chymotrypsin, pig liver esterase (P.L.E.), and several lipases, especially the lipase from *Candida cylindracea* and porcine pancreatic lipase (P.P.L.). However, the lipase from *Pseudomonas fluorescens* is becoming increasingly popular.[22]

The mechanism of action of the above hydrolases involves a classical acyl-enzyme intermediate (Scheme 1.20).[58]



Scheme 1.20

They each have a catalytic serine residue which can be inactivated by organic fluorophosphates (Scheme 1.21).[59]



DIPF

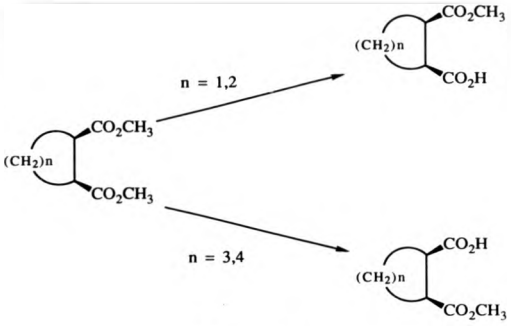
DIPF = diisopropylphosphofluoridate

Scheme 1.21

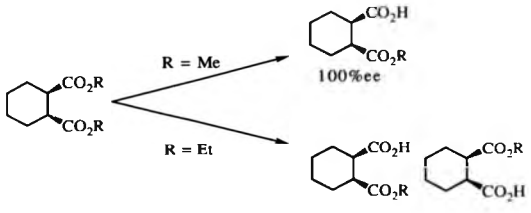
1.3.1. ESTERASES

Pig liver esterase (P.L.E.) [EC.3.1.1.1] consists of a series of isozymes with similar specificities.[60] One of the best applications of P.L.E. is the hydrolysis of *meso* compounds. *Meso* compounds are superb substrates from a practical point of view in so much as the enzyme reaction can proceed in theory to quantitative yield, with complete stereospecificity (100% yield, 100%ee).[17,18]

e.g. (Schemes 1.22 and 1.23).



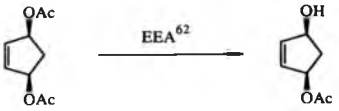
Scheme 1.22



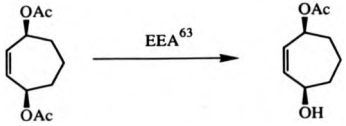
Scheme 1.23

Recently Jones has proposed a cubic space model to account for these previously mystifying reversals of stereochemistry.[61]

Electric eel acetylcholinesterase (E.E.A.) [EC.3.1.1.7] has also been used for the stereospecific hydrolysis of *meso* compounds. Here a remarkable change in stereospecificity was observed (Scheme 1.24).[62,63]



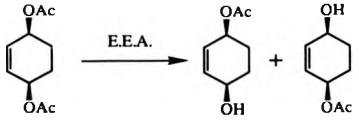
94% yield,
99%ee



39% yield,
100%ee

Scheme 1.24

This reversal has a parallel in a reaction described in Chapter five of this thesis, namely the hydrolysis of *cis*-3,6-diacetoxy cyclohexene (2) which resulted in racemic hydroxyacetate (3) (Scheme 1.25).



(2) (3) 50 : 50

Scheme 1.25

1.3.2 LIPASES

Lipases (Triacylglycerol lipase; Triacylglycerol acylhydrolase [EC.3.1.1.3]) are commonly used biocatalysts. In nature their function is to hydrolyse triacyl glycerides (4) to produce long

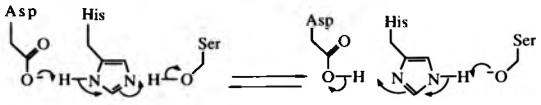
chain fatty acids (5) and the parent glycerol (6) (Scheme 1.26).[64]

(4) (6) (5)

Scheme 1.26

They act at oil/water interfaces and are therefore well suited to hydrolysing water-insoluble substrates. Bacterial lipases are extracellular and are therefore easy to purify in bulk quantities. In 1985 they represented 3% of the World enzyme market.[65] They are used in the food industry to modify the properties of oils and fats.[65]

Recently, two papers have appeared in *Nature*, both describing the X-ray structure of lipases.[66,67] Both lipases appear to have a catalytic triad akin to the charge relay system of the serine proteases (Scheme 1.27).[68]

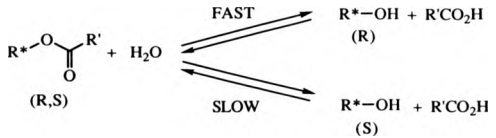


Scheme 1.27

The active serine is buried inside the enzyme and is protected by a loop of amino acid residues. This loop has to move to allow access by the substrate to the active site.

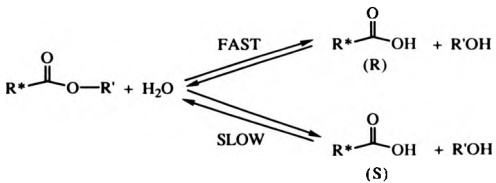
As well as hydrolysing *meso* compounds lipases have been extensively applied to resolve racemic mixtures into their antipodes by the stereoselective hydrolysis/esterification of one enantiomer. In the following cases the (R) enantiomer is the faster reacting (Schemes 1.28 and 1.29).[22]

(A) Resolution of racemic alcohol/achiral acid ester:



Scheme 1.28

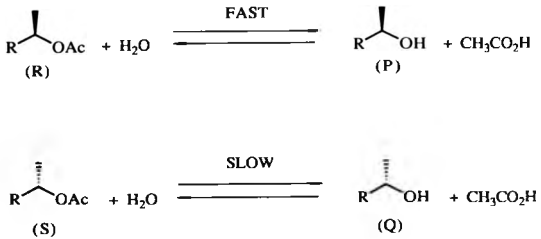
(B) Resolution of racemic acid/achiral alcohol:



Scheme 1.29

The degree of enantioselectivity a particular enzyme expresses with respect to a given substrate can be quantified, by determining the E value as derived by Sih.[69]

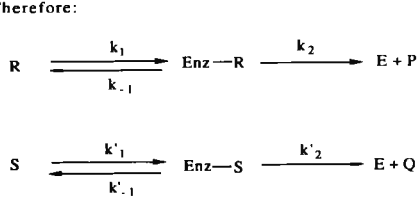
For a given racemic mixture, let us consider esters of racemic alcohols. Assume that a particular enzyme hydrolyses the (R) enantiomer preferentially (Scheme 1.30).



R = alkyl > CH_3

Scheme 1.30

Therefore:



Since the reaction is carried out in water (55 molar) at low substrate concentrations, hydrolysis is essentially irreversible.

Under Michaelis-Menten conditions (i.e. [S] -> 0, $k_{-1} >> k_2$, and $k'_{-1} >> k'_2$), the relative rates of formation of P/Q (the enantiomeric ratio or E value) is given by:

$$E = \frac{\ln\left(\frac{[R]}{[R]_o}\right)_R}{\ln\left(\frac{[S]}{[S]_o}\right)_S} = \frac{k_2\left(\frac{k_1}{k_{-1}}\right)_R}{k'_2\left(\frac{k'_1}{k'_{-1}}\right)_S} = \frac{\left(\frac{k_{cat}}{K_m}\right)_R}{\left(\frac{k_{cat}}{K_m}\right)_S}$$

Where:

[R] = concentration of remaining (R) substrate at time t.
$[R]_o$ = Initial concentration of (R) at time = 0

However, if the formation of the enzyme substrate complex is rate limiting (i.e. all active sites of the enzyme are saturated ([S] > $2K_m$):

i.e. $k_2 >> k_1$
and $k'_2 >> k'_1$

Then $E = (k_1/k'_1)$

The difference in the free energy of the two diastereomeric transition states is related to the E value:

$$\Delta\Delta G^{\#} = -RT \ln E$$

if, for example $\Delta\Delta G^{\#} = 12$ kJmol^{-1} (3 kcalmol^{-1})
then $E = 100$

The E value describes a given resolution for a given enzyme. The higher the E value the better the stereoselectivity. Sih established that the E value can be determined by measuring two parameters $\%ee_{(p)}$ and $\%ee_{(sm)}$ at the same conversion (c), (p = product, sm = residual starting material).
The conversion (c) can be physically determined, by g.l.c., or h.p.l.c. for example, or more readily from the equation:[69]

$$c = \frac{ee_{(sm)}}{ee_{(sm)} + ee_{(p)}}$$

The E value is given by:

$$E = \frac{\ln([1-c][1-ee_{(sm)}])}{\ln([1-c][1+ee_{(sm)}])}$$

This allows the relationship between %ee(sm) and c and between %ee(p) and c to be plotted (Figure 1.1 and 1.2).[22]

FIGURE 1.1 Plot of the relationship between %ee (starting material) and % conversion for various E values.[22]

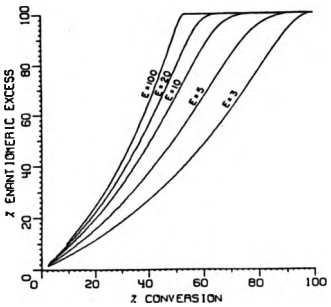
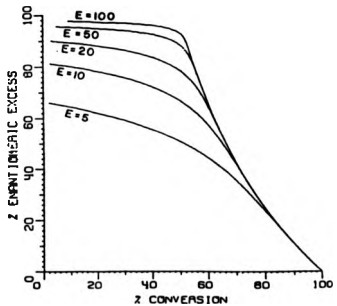


FIGURE 1.2 Plot of the relationship between %ee (product) and % conversion for various E values.[22]



Thus the E value is independent of degree of conversion (for a given set of conditions). One can simply determine from the two graphs when to terminate the reaction to obtain the desired enantiomer with the desired optical purity.

From inspection of the graphs several points can be made:

(1) Even for a large E value the reaction must be terminated at or before c=0.5 if optically pure product is desired.

(2) For E > 100 the reaction is essentially completely stereoselective.

(3) With low E values (for example E=5) optically pure starting material can be obtained, but at the expense of chemical yield.

(4) For practical purposes the E value should be > 20.

(5) For E>100 an accurate determination of E is difficult to achieve.[70,81]

For a given resolution the enantioselectivity can be improved in several ways:

(1) Modification of the substrate (e.g. Table 1.4).[71]

Table 1.4 Substrate modification of racemic ester (7) leads to an increase in enantioselectivity.

(+,-)(7)

PPL = porcine pancreatic lipase

R	Conversion, c	E
CH_3	0.60	4
C_2H_5	0.60	11
C_3H_7	0.60	13
C_4H_9	0.60	16
C_5H_{11}	0.60	16

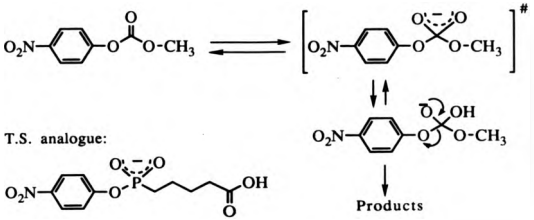
(2) Screening of other biocatalysts to find an enzyme with a larger E value.[22]

(3) Recycling the product.[22] This has successfully been employed with E values as low as 10. The product after initial exposure to the enzyme is isolated, esterified and re-exposed to the same enzyme.

(4) The enzyme can be redesigned by enzyme engineering to produce an enzyme with more desirable properties. For example using site directed mutagensis the protease subtilisin was converted into a more stable form by replacing a readily oxidisable methionine residue.[72,73] This technique holds the promise of altering the properties of an enzyme at will e.g. pH profile, temperature stability, substrate specificity, etc. However

this represents a labour intensive, highly skilled task. In 1986 it was projected to cost US$1,000,000 per enzyme.[72]

(5) Catalytic antibodies can be raised to a mimic of the reaction's putative transition state (e.g. Scheme 1.31).[74] For the hydrolysis:



Scheme 1.31

If a judicious choice of stable T.S. analogue is selected then it is possible to achieve 10^3-10^5 rate accelerations when compared to the base-catalysed hydrolysis reaction. This technique is still in its infancy but it offers the reward of "enzyme like catalysts with tailored specificities".[74]

(6) Enzyme immobilisation to allow easier separation of product from enzyme.[55]

(7) Utilising the enzyme in the reverse/esterification direction. This can be achieved by using biphasic systems, or even better, in very low water systems. The technique is easily applicable in a standard chemical laboratory. It circumvents several of the problems associated with enzymes in aqueous solutions:[21]

(A) Substrates are usually more soluble in organic solvents.

(B) Similarly, substrates are often more stable in organic solvents.

(C) Separation of insoluble enzyme and product is trivial.

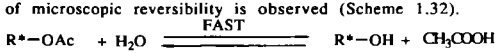
(D) The enzymes have the potential to be recycled.

(E) Many enzymes are, surprisingly, more stable in organic solvents, than in an aqueous environment.[65]

(F) The pH of the system no longer has to be regulated.

(G) In several cases increased enantioselectivity is observed.[22]

The principle of microscopic reversibility states that the enzyme mechanism must be the same in the forward direction as in the reverse direction (for a given set of conditions).[75] So, if the enzyme is operating in the reverse, esterification, direction it can be deduced that the mechanism will be the exact reversal of the hydrolytic method. Therefore, it follows that if one enantiomer is hydrolysed faster than the other, then the same enantiomer will be esterified faster, when the enzyme is operated in the reverse direction. This argument holds for enzymes whose mechanism do not alter when the bulk media is changed from aqueous to an organic solvent. i.e. if the principle of microscopic reversibility is observed (Scheme 1.32).

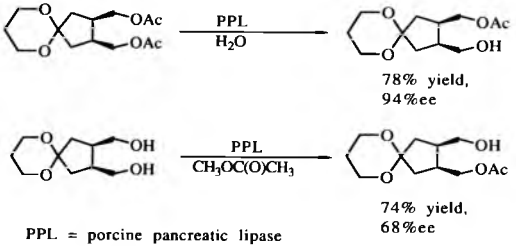


(R)

(S)

Scheme 1.32

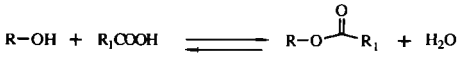
This allows access to either enantiomer by simply altering the solvent and the nature of the starting material (Scheme 1.33).[76]



PPL = porcine pancreatic lipase

Scheme 1.33

Esterification reactions can be carried out as mentioned above in biphasic systems or using the free acid as acyl donor in an organic solvent such as isooctane. The problems with such systems is that as the reaction proceeds water is produced. The enzyme can now hydrolyse the ester and hence an equilibrium concentration of ester will be achieved (Scheme 1.34).

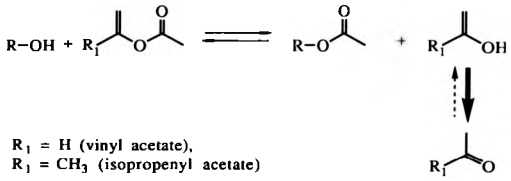


Scheme 1.34

Furthermore, as the reaction proceeds to equilibrium the enantioselectivity can decrease, this can be simply explained. If for example, the (R) enantiomer is esterified preferentially then as the reaction proceeds the levels of the (R) ester will increase.

However, as the reaction approaches equilibrium the (R) ester will be hydrolysed preferentially. In the worst case scenario racemic ester will achieved, if the reaction is left long enough at equilibrium.[77]

The problem has been recently circumvented. Novel acylating agents have been introduced,[80] which effectively mean that the esterification is operated under irreversible conditions.[78,79] The acylating agents are enol acetates, the ester product being produced along with an enol which quickly tautomerises to an inactive aldehyde or ketone (Scheme 1.35).



R_1 = H (vinyl acetate),
R_1 = CH_3 (isopropenyl acetate)

Scheme 1.35

Since no equilibrium is set up the faster reacting enantiomer is not reversibly hydrolysed, and the enantioselectivity or enantiospecificity will not decrease with conversion.

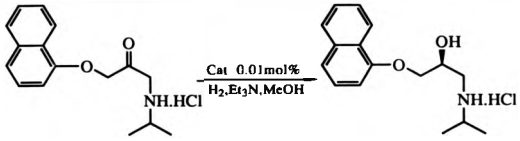
CHAPTER TWO

Chemoenzymatic synthesis of optically pure β-blocker.

2.1 INTRODUCTION

Beta-adrenoceptor antagonists (β-blockers) are extremely lucrative pharmaceutical drugs. The worlds leading β-blocker is I.C.I.'s Tenormin (atenolol) which generated £530 million in 1987.[84] One of the first β-blockers was Inderal (propranolol), which in 1987 was the worlds number sixteen drug and accounted for £230 million.[84] The U.S. β-blocker market has been estimated to rise at an annual rate of 4% to be worth 1,281,000,000 U.S.$ in 1992.[85] In the United States alone sixty million people have high blood pressure (hypertension).[85] Beta-blockers reduce hypertension, relieve angina and a host of other problems, by countering the action of sympathetic amine release (for example adrenalin) which elicits a "fight or flight" response from the individual.[86]

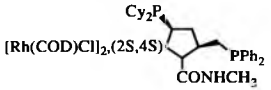
The biological activity lies in the (S)-enantiomer.[87,88] Chemical routes to both enantiomers have been published. For example, starting from sugars, both (R)- and (S)-practolol has been synthesised from D-mannitol.[89] Syntheses starting from D-mannitol usually proceed with 4 to 15% overall yield.[90] Better chemical routes start from chiral acetals.[90] Recently an efficient transition metal-catalysed asymmetric hydrogenation of an amino ketone (7) led directly to (S)-propranolol (8) (100% yield, 91%ee).[91] The product was homochiral after one recrystallisation (Scheme 2.1).



(7)

(8), 100% yield, 91%ee. After one recrystallisation: 92% yield, *ca.* 100%ee

catalyst:



Scheme 2.1

Enzymatic routes to (S)-propranolol (8) have been demonstrated. The lipase from *Pseudomonas fluorescens* catalysed the resolution of racemic aryloxyacetaldehyde cyanohydrin acetates (9) (Scheme 2.2).[92]

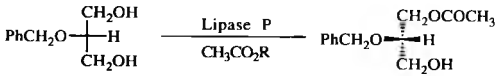
(9)

(10) 40% yield, 88%ee.

Scheme 2.2

The residual starting material (10) was converted to optically active (S)-propranolol (8) in two steps.

Lipase P, also from *Pseudomonas fluorescens*, stereospecifically esterified a *meso* 1,3-diol (11) (Scheme 2.3).[93]

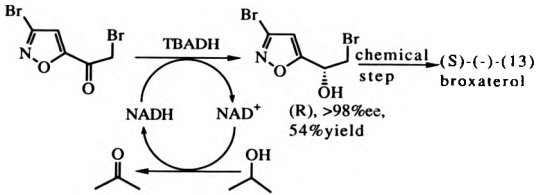


(11)

(S)-(12)
92% yield,
92% ee.

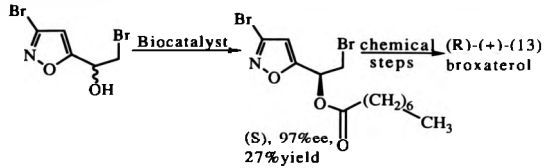
Scheme 2.3

The product (12) was converted in seven steps to (S)-propranolol (8). Recently chemoenzymatic routes to both enantiomers of the β-blocker broxaterol (13) have been published[94] (Schemes 2.4 and 2.5).



TBADH=Alcohol dehydrogenase from *Thermoanaerobium brockii*

Scheme 2.4



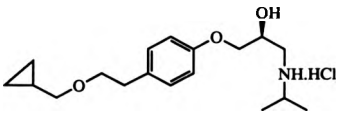
Biocatayst:
Lipase P (*ex Pseudomonas fluorescens*),
Trifluoroethyl octanoate,
n-hexane/benzene : 9/1,
4Å molecular sieves.

Scheme 2.5

Some other enzymatic and non enzymatic asymmetric routes to β-blockers are given in references.[91,92,93]

2.2 AIMS

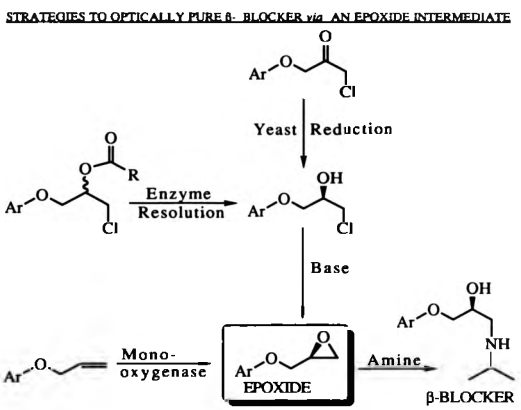
Our target molecule was the chemoenzymatic synthesis of the (S)-enantiomer of the β-blocker (14) (Betaxalol):



(14)

which is currently being marketed by the French company Synthelabo, as its corresponding HCl salt. Our strategy involved the enantiospecific or enantioselective formation of an epoxide

intermediate which could be subsequently, chemically, converted into the target molecule (Scheme 2.6).

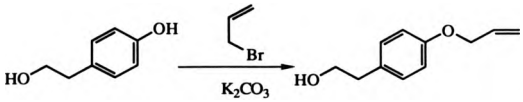


Scheme 2.6

2.3 MONOOXYGENASE APPROACH

Initially our approach was to try to epoxidise enantiospecifically a prochiral double bond using a monooxygenase. This was to be followed by regiospecific nucleophilic ring opening of the epoxide with the requisite amine to furnish the desire β-blocker (see Scheme 2.6). At Warwick several monooxygenase systems were available for study. After discussion with Professor H. Dalton (Department of Biological Sciences, University of

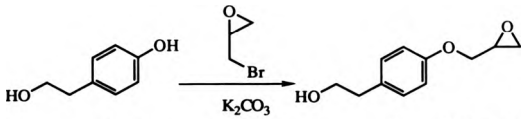
Warwick) it was decided that the initial alkene should be compound (16). This was synthesised by refluxing together 2-(4'-hydroxyphenyl)ethanol (15), allylbromide and potassium carbonate in butanone, following a recipe supplied by, the then, SmithKline and French Research Ltd. (Scheme 2.7).



(15) (16) 87% yield

Scheme 2.7

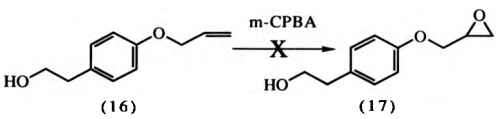
The target compound (17) was then synthesised in an analogous way so that a spectroscopic and chromatographic standard was in hand (Scheme 2.8).



(15) (17) 92% yield

Scheme 2.8

This circumvented earlier problems since the alkene (16) was not epoxidised directly with *meta*-chloroperbenzoic acid (m-CPBA) (Scheme 2.9).



(16) (17)

Scheme 2.9

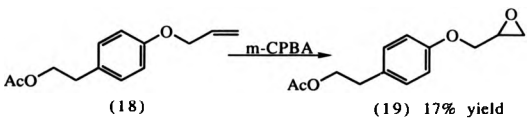
Conditions:

(1) CH_2Cl_2, room temperature.

(2) CH_2Cl_2, 50 °C.

(3 CH_2ClCH_2Cl, radical inhibitor,[95] reflux.

Following protection of the primary hydroxyl group of (16) as the acetate (18), epoxidation of which was possible to give (19), but in only low yield (Scheme 2.10).



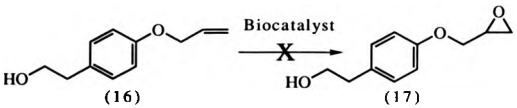
(18) (19) 17% yield

Scheme 2.10

The first enzyme system chosen for study was the readily available methane monooxygenase (MMO) from the strict methanotroph *Methylococcus capsulatus* (Bath). The enzyme is water soluble and a crude enzyme preparation was donated by Dr J. Green (Department of Biological Sciences). The crude preparation contains all of the cells enzymes, including of course MMO. MMO is composed of three sub-units, all of which are essential for catalytic activity. The optimal pH and temperature are 7 and 45 °C respectively. MMO is only stable for a short

time at 45 °C. Furthermore, the extract contains an NADH oxidase which quickly oxidises the added external NADH. Therefore, as a rule, all biotransformations must be complete after three minutes, at 45 °C.[96]

No epoxidation of alkene (16) was observed (the reaction was followed by g.l.c.). This is probably not surprising as oxidations mediated by MMO have only been reported for molecules as large as C_8[96] whilst alkene (16) is a C_{11} compound (Scheme 2.11).



(16) (17)

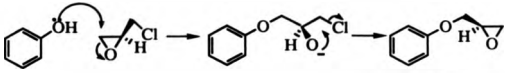
Biocatalyst:
Crude cell extract of *Methylococcus capsulatus* (Bath),
45 °C,
Phosphate buffer 20mM, pH 7, NADH.

Scheme 2.11

In the synthesis of epoxide (17) racemic epibromohydrin was used. Accordingly it was decided to attempt to epoxidise allyl halides enantiospecifically, followed by incorporation into the synthesis of the β-blocker.

Mc Clure, Arison and Baldwin have shown that the mode in which epichlorohydrin and related systems are opened by nucleophilic attack can be controlled both by the reaction conditions and by the leaving group involved (Scheme 2.12).[97]

(A) INVERSION OF CONFIGURATION



90% ee

Conditions:
K_2CO_3, Acetone, reflux.

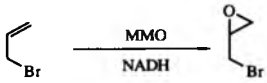
(B) RETENTION OF CONFIGURATION

96% ee

Conditions:
Preformed anion (NaH/THF).

Scheme 2.12

Epibromohydrin was epoxidised by MMO in approximately 5% yield, by g.l.c. (Scheme 2.13).



Scheme 2.13

As well as epibromohydrin, allyl alcohol was also produced (identified by co-injection and G.C.-M.S.). This was shown to be attributable to non-enzymic hydrolysis in the buffer. In an attempt to reduce the amount of allyl alcohol generated, allyl chloride was epoxidised by MMO (5% yield). However allyl

alcohol was again produced in appreciable amounts. In control experiments:

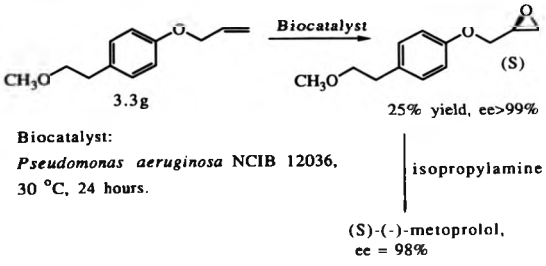
$$\frac{\text{Rate of buffer hydrolysis of allyl bromide}}{\text{Rate of buffer hydrolysis of allyl chloride}} = \frac{3}{1}$$

Also, the product epichlorohydrin was racemic as determined using a chiral capillary g.l.c. column (50m x 0.22mm with 0.125M Nickel bis [3-heptafluorobutyryl-(1R)-camphorate] on an OV101 support). The results are in broad agreement with work carried out by Golding who used MMO to epoxidise propene. Golding observed a 2% conversion to propene oxide which he determined to be racemic.[98] Two more bacterial strains were screened for their ability to epoxidise allyl chloride (Table 2.1).

Table 2.1 Attempted enzymatic epoxidation of allyl chloride.

Microorganism	Epoxidation?	
	Whole cells	crude extract
Pseudomonas putida (containing a tol plasmid)	X	X
Pseudomonas butanovora	X	1%

However at the time Shell International Research filed two European patents in which the aims of my project were successfully delineated. For example (Scheme 2.14):[99,100]

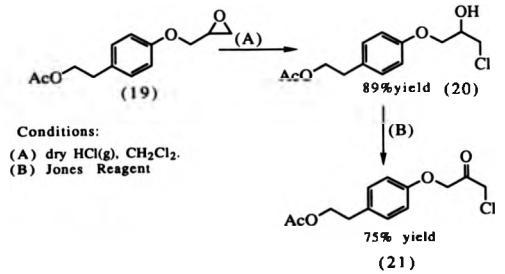


Scheme 2.14

Accordingly, at this stage it was decided to change strategy.

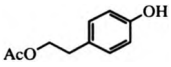
2.4 OXIDOREDUCTASE APPROACH

The ketone (21) was synthesised (Scheme 2.15), using standard methods. The synthesis is based on a similar procedure which started from 3-phenoxy-1,2-epoxypropane.[101]



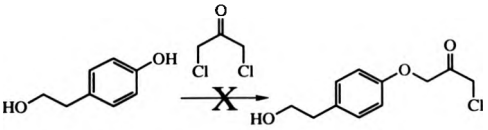
Scheme 2.15

It was found, by experiment, that exactly 1.5 mol Cr^{+6} per mole of substrate (20) was required for optimum yield of the crystalline α-chloroketone (21). Deviation from 1.5 mol Cr^{+6} per mole of substrate led to production of phenolic compound (22) which proved to be difficult to separate from chloroketone (21):



(22)

Direct synthesis of α-chloroketone (22) by reacting together 2-(4'-hydroxyphenyl)ethanol (15) and 1,3-dichloroacetone under various basic conditions was, unfortunately, unsuccessful (Scheme 2.16).



(15) (22)

Scheme 2.16

Conditions:

(1) K_2CO_3, acetone, reflux.

(2) K_2CO_3, KI(cat), acetone, reflux.

(3) K_2CO_3, 18-crown-6, acetone, reflux.

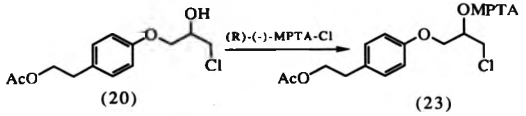
(4) K_2CO_3, 18-crown-6, DMF, reflux.

(5) Tetramethylammoniumhydroxide, dimethylacetamide,R.T.

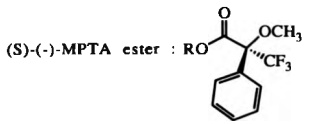
(6) KF on celite,[102] THF, R.T.

(7) KF on celite,[102] THF, reflux.

The acetylated α-chloroketone (21) was then reduced by various microorganisms. The first was baker's yeast which gave product (20) in 27% isolated yield but with only 25%ee. The %ee was determined by converting (20) into its corresponding (S)-(-)-Mosher's ester (23),[103] followed by ^1H n.m.r. (400 MHz) analysis (Scheme 2.17).



(20) (23)



Scheme 2.17

The chemical shifts for the two diastereomeric singlets attributable to the methoxy protons were completely separated at δ = 3.60 and 3.55 p.p.m.

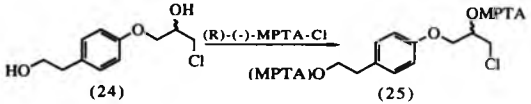
A sample of the chloroketone (21) was sent to Professor G. Morris (Department of Botany and Microbiology, The University College of Wales, Aberystwyth). One of his co-workers (E. Tidswell) studied the reduction of (21) using a series of microorganisms. Purification and chiral analysis were carried out at Warwick. In most cases the major product was the desacetoxy chlorohydrin compound (24) (Table 2.2).

Table 2.2 Microbiological reduction of chloroketone (21).

(21) (24)

Microorganism	%ee	Diastereomer A/B ?	% yield
Lactobacillus brevis	29	A	17
Clostridium pasteurianum	60	B	19
Clostridium tyrobutyrium LA1	60	B	13
Clostridium kluyveri	88	B	18

The %ee was determined indirectly by making the *bis*-(S,S)-Mosher's ester (25) (Scheme 2.18)[103] followed by chiral analysis using ^{1}H n.m.r. (270 MHz).



(24) (25)

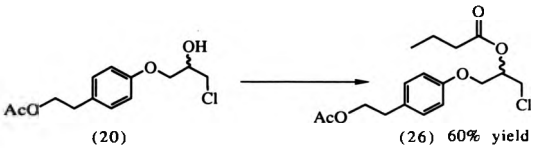
Scheme 2.18

The diastereomeric signals associated with the methoxy protons are baseline resolved at δ = 3.60, 3.55 and 3.40 p.p.m. When racemic chlorohydrin (24) was converted into diester (25) the relative ratio of these sets of signals were 1.5 : 1.5 : 3. The desacetoxy α-chlorohydrin (24), unfortunately, is a liquid so enhancement of the ee by recrystallisation was not possible. As well as the oxidoreductase approach a concurrent approach to chlorohydrin (20) had been undertaken using hydrolytic

enzymes. It should be borne in mind that the maximum yield of optically pure chlorohydrin (20) permissible with the resolution approach is 50%.

2.5 HYDROLYTIC APPROACH

The butyrate (26) of the chlorohydrin (20) was synthesised (Scheme 2.19).

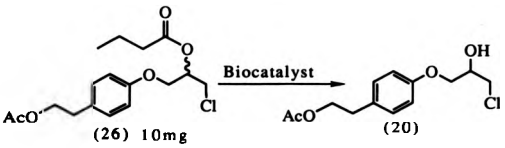


(20) (26) 60% yield

Conditions:
Butyric anhydride, DMAP(cat.), CH_2Cl_2, pyridine.

Scheme 2.19

Ten enzymes were screened for their ability to hydrolyse the racemic substrate (26) (Scheme 2.20).



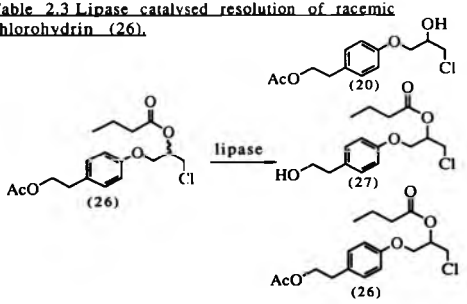
(26) 10mg (20)

Biocatalyst:
Lipase/esterase *ca.* 10mg.
Phosphate buffer, pH 7, 20mM, 1ml.
Acetone, 0.1ml.

Scheme 2.20

The reactions were followed by t.l.c. All enzymes accepted (26) as a substrate. Two enzymes were selected for further study on the basis that the reactions appeared to be proceeding to 50% conversion and that the reactions appeared to be both chemio- and regioselective i.e. the butyrate group, not the acetate, was hydrolysed. The two enzymes selected were the lipases from *Mucor* species (lipase M *ex* Amano) and *Rhizopus javanicus* (lipase N *ex* Amano). The reactions were performed exactly as above but on a 2X scale. After work-up the reaction mixture was separated into its individual components by flash chromatography. The results are shown below (Table 2.3).

Table 2.3 Lipase catalysed resolution of racemic chlorohydrin (26).



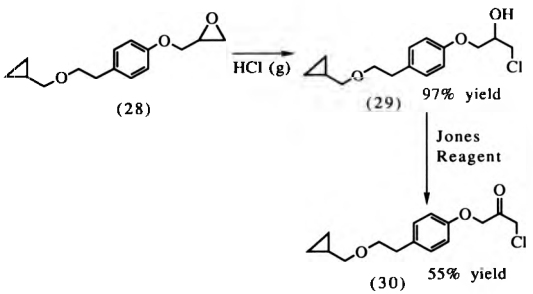
Lipase	(20) / %yield, %ee	(27) / %yield	(26) / % yield
Mucor species.	20, 90.	8	65
Rhizopus javanicus	36, >98	15	43

The %ee of chlorohydrin (20) was determined in the usual manner by synthesising the Mosher's ester derivative (23).[103] Although the reaction appeared to be highly stereoselective in the case of the lipase from *Rhizopus javanicus* the reaction was not completely chemio- and regioselective. Considerable hydrolysis of the acetate function occurred (15%). Hydrolysis of the acetate group was causing considerable concern. Accordingly, it was subsequently decided to synthesise the fully protected β-blocker precursor. In this case the primary hydroxyl group was protected as the hydrolytically stable cyclopropylmethyl ether instead of employing the labile acetate group. As well as being inert to hydrolysis the cyclopropylmethyl group had the advantage of being closer in chemical structure to the ultimate target structure. Therefore less post-enzymatic modification of the desired product would be required. However, fewer β-bocker analogues can be synthesised, since realistic structural modifications can only take place on the phenolic side of the molecule. This situation could be altered, if structural analogues were desired, by protecting the primary hydroxyl group with a chemically readily removable protecting group, but hydrolytically stable protecting group, for example a trityl ether could be synthesised.

2.6 CYCLOPROPYLMETHYL - FULLY PROTECTED PRECURSOR

The synthesis of α-chloroketone (30) (Scheme 2.21) follows closely the previous synthesis of compound (21) (see Scheme 2.15).[101] The starting epoxide (28) was donated by Dr M. B.

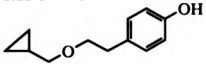
Mitchell (SmithKline Beecham Ltd). Epoxide (28)[104] was smoothly opened to chlorohydrin (29) by the addition of dry, gaseous, hydrogen chloride. The reaction proceeded in nearly quantitative yield. The chlorohydrin was oxidised to the desired α-chloroketone (30) with Jones reagent.[105]



(28) (29) (30)

Scheme 2.21

Other oxidising regents were used to try to optimise the yield of (30). Both Pyridinium dichromate (PDC) and pyridinium chlorochromate (PCC) oxidised (29), but at a considerably lower rate. Side products were observed by t.l.c. As experienced with compound (21), compound (30) is unstable to silica gel, breaking down to the phenol (31) (established by isolation of (31) and comparison with an authentic sample). The maximum purity of (30) obtained was 97% (by n.m.r.). It is however, quite stable when stored in a freezer at -20 °C.

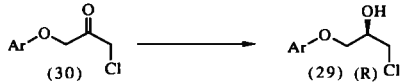


(31)

2.7 MICROBIOLOGICAL REDUCTION OF α-CHLORO KETONE (30)

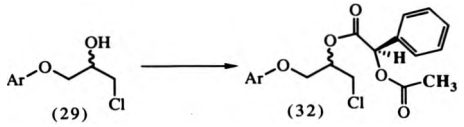
Compound (30) (50-100 mg) was reduced by several microorganisms at a substrate concentration of 1mg/ml (Table 2.4). For entries #1-3 raw baker's yeast (Sainsburys) was used. For entries #4-8 the reduction was carried out by E. Tidswell (University of Aberystwyth). For entries #9-14, the yeasts were cultivated from agar slopes in YM media at 30 °C for 24 hours, then a one in ten dilution was made to fresh YM media, which was subsequently incubated for 24 hours before substrate was added. The reduction were then carried out at 30 °C on shaker-incubators. The reactions were monitored periodically by t.l.c. The results are given below (Table 2.4):

Table 2.4 Microbiological reduction of α-chloroketone (30).



Entry #	Microorganism	Product/ %yield	Product/ %ee	R/S
1	Bakers yeast (3mg/ml)	39	42	R
2	Bakers yeast (1mg/ml)	63	47	R
3	Bakers yeast (+allyl alcohol 1mg/ml)	38	52	R
4	*Clostridium pasteurianium*	9	67	R
5	*C.tyrobutyricum* LA1	3	27	R
6	*C.tyrobutyricum* LA1	8	44	R
7	*C.tyrobutyricum* TM(V)	3	39	R
8	*Lactobacillus brevis*	32	28	S
9	OY P1	26	35	R
10	OY G2	20	50	R
11	*C. guilliermondii* NCYC 1399	73	34	R
12	*S. cerevisiae* NCYC 240A	50	38	R
13	"Ogihara (1)"	22	38	R
14	OY B2	16	36	R

The % ee was determined by synthesising the (R)-*O*-acetyl mandelic ester (32) (Scheme 2.22). This was achieved by coupling the alcohol (29) with (R)-*O*-acetyl mandelic acid using 1,3-dicyclohexylcarbodiimide (DCC) as the coupling reagent:[106,107]



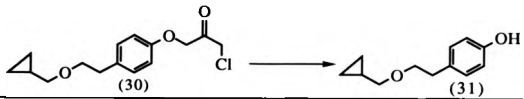
Conditions:
DCC, DMAP, CH_2Cl_2, (R)-*O*-Acetyl mandelic acid, -11°C to RT.

Scheme 2.22

Compound (32) was isolated by flash chromatography. The enantiomeric excess was then determined at ^1H n.m.r. (400 MHz). This gave beautiful splittings for all the diastereomeric sets of signals. The two sets of protons in **bold** in the above diagram (Scheme 2.22) were especially diagnostic. The diastereoisomeric acetyl methyl protons gave signals at δ = 2.20 and 2.18 p.p.m., whereas the diastereoisomeric methine protons gave signals at δ = 5.92 and 5.91 p.p.m. Both sets of signals were baseline resolved and so an inbuilt check on the %ee was available, thereby minimising errors caused by the possibility of overlapping impurity peaks. (For examples of spectra see Section 2.11, of this chapter. For determination of the stereochemistry see Section 2.10, also in this chapter.)

From perusal of Table 2.4 it can be seen that the %ee obtained are only moderate to good whilst the yields of halohydrin (29) obtained, are at best 60%, but in most cases the yield is considerably lower. The low yield was usually observed in those cases where the time taken for the microorganism to reduce the ketone was greater than one day, because, as well as microbiological reduction, there was simultaneously a slow breakdown of the ketone (30) to phenol (31). In order to try to establish why this breakdown occured several test reactions were constructed and performed (Table 2.5):

Table 2.5 Why is the chloroketone (30) unstable?



Conditions:	Time/ days	^{1}H n.m.r. ratio of Ketone(30) : Phenol(31)	
Yeast medium (pH 6.2)	12	17	83
MeOH/H_2O/HCl/air/$h\nu$	12	66	34
MeOH/H_2O/argon/dark	4	87	13
MeOH/H_2O/argon/$h\nu$	4	85	15
MeOH/H_2O/air/dark	4	92	8
MeOH/H_2O/air/$h\nu$	4	90	10

The ketone appears to be relatively stable in neutral solutions (bottom four examples in Table 2.5). Also, an ^{1}H n.m.r. sample of α-chloroketone (30) in deuteriochloroform showed only 7% phenol (31) content after two weeks. Accordingly, it can be concluded that the breakdown is not an oxidative process. The ketone (30) breaks down on silica gel and in the yeast medium

(YM). Both of these environments are acidic. However, relatively little breakdown to phenol (16) was observed when chloroketone (30) was placed in an acidic solution. It was concluded that breakdown of chloroketone (30) to the phenol (31) in the yeast medium is catalysed by a component of the medium. However, the nature of this catalysis is unknown.

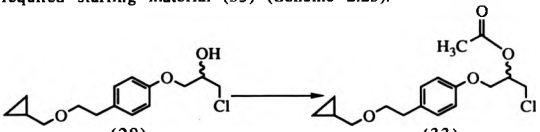
Baker's yeast reduction proceeded to give the (R)-chlorohydrin (29) in moderate to good yield. Changing the substrate concentration from 3mg/ml to 1mg/ml[52] considerably increased the yield, but only marginally increased the ee. Addition of allyl alcohol[53] only appeared to inhibit the rate of reduction. The ee increased slightly, but not significantly.

The problems with the substrate sensitivity and the fact that no synthetically useful results were obtained promoted a return to the use of hydrolytic enzymes, in an attempt to resolve a racemic mixture into its enantiopodes, followed by chemical conversion to the desired β-blocker. This was successfully achieved.

2.8 RESOLUTION ROUTE TO OPTICALLY PURE β-BLOCKER

2.8.1 HYDROLYTIC APPROACH

The racemic chlorohydrin (29) was acetylated to give the required starting material (33) (Scheme 2.23).

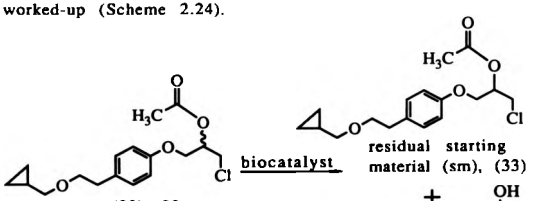


(29) (33)

Conditions:
Acetic anhydride, pyridine, CH_2Cl_2, DMAP.
87% yield

Scheme 2.23

A screen of ten hydrolytic enzymes was set up for their ability to enantioselectively hydrolyse one enantiomer of ester (33). The reactions were followed qualitatively by t.l.c. Reactions that appeared to be consistently 50% complete after four days were worked-up (Scheme 2.24).

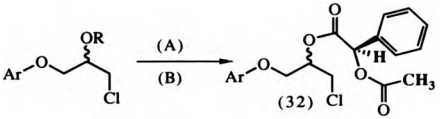


(33), 20mg

Biocatalyst:
Enzyme *ca.* 10mg
Phosphate buffer 100mM, pH 7, 1ml.

Scheme 2.24

The residual starting material (sm) was separated from the product (p) by flash chromatography. The %ee of the product (29) (%ee(p)) was established, as before, by making the (R)-*O*-acetyl mandelic ester (32).[106,107] In order to determine the E value for the reaction the %ee of the starting material (%ee(sm)) was determined. This involved, base catalysed hydrolysis to the chlorohydrin (29) (no epoxide (28) was observed by either t.l.c. or ^1H n.m.r.), followed by derivatisation to give the (R)-*O*-acetyl mandelic ester (32) (Scheme 2.25).[106,107]



If R = H, (29)
(A) = DCC, DMAP (cat.), CH_2Cl_2, (R)-*O*-Acetyl mandelic acid.
(B) not applicable.
If R = $C(O)CH_3$, (33)
(A) = MeOH, K_2CO_3(2 eq.), -11 °C to 0 °C.
(B) = DCC, DMAP (cat.), CH_2Cl_2, (R)-*O*-Acetyl mandelic acid.

Scheme 2.25

The ee was then determined by ^1H n.m.r (400 MHz) as described previously (Section 2.7). The results are as given in the following table (Table 2.6):

Table 2.6 Enzymatic screen for the resolution of racemic acetate (33).

Lipase	Conversion, c	E
Aspergillus niger	0.72	4
FAP-15	0.26	2
Mucor species	0.32	4
Rhizopus javanicus	0.40	2
Porcine pancreatic lipase	0.40	5
Pseudomonas fluorescens	**0.63**	**14**
Pig liver esterase	0.29	1

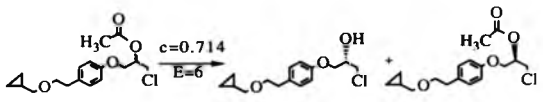
where: $E = \dfrac{\ln([1-c][1-ee_{(sm)}])}{\ln([1-c][1+ee_{(sm)}])}$

and $c = \dfrac{ee_{(sm)}}{ee_{(sm)} + ee_{(p)}}$

Derivation of the E value and extent of conversion, c, is given in Section 1.3.2 and reference.[22] In each case the (S)-enantiomer was hydrolysed faster than the (R)-enantiomer, except for the lipase from *Aspergillus niger* where the stereoselectivity in the hydrolysis was the opposite of that with the other enzymes screened. (I.e. the (S)-alcohol was preferentially formed). Determination of the absolute stereochemistry is given in Section 2.10 of this chapter.

From Table 2.6 the lipase from *Pseudomonas fluorescens* (*ex* Biocatalysts Ltd) was selected as the most enantioselective (E = 14, c = 0.63, %ee(p) = 57, %ee (sm) = >97). This reaction was

scaled-up to a one gram scale (X50) (Scheme 2.26), employing the same conditions as above (see Scheme 2.24):



(33), 1g

(S)-(+)-(29)
19% yield
36%ee

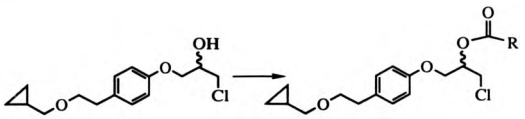
(R)-(-)-(33)
68% yield
90% ee

Scheme 2.26

The enantioselectivity as expressed by the E value was just about synthetically useful. It was decided to try improve the enantioselectivity by the well known method of substrate modification.[71]

2.8.2 SUBSTRATE MODIFICATION

From the enzyme screening experiments the lipase from *Pseudomonas fluorescens* had been selected. Two modifications to the substrate were made both designed to increase the length of the acyl group (Scheme 2.27).



(29)

R = CH_3, (33), 87% yield.
R = C_3H_7, (34), 90% yield.
R = C_5H_7, (35), 85% yield.

Conditions:

R = CH_3, (33), acetic anhydride, pyridine, DMAP, CH_2Cl_2.

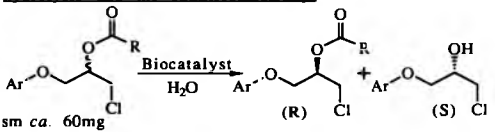
R = C_3H_7, (34), butyric anhydride, pyridine, DMAP, CH_2Cl_2.[108]

R = C_5H_7, (35), hexanoic acid, DCC, DMAP, CH_2Cl_2.

Scheme 2.27

Both (34) and (35) were good substrates for the lipase from *Pseudomonas fluorescens*. They were both hydrolysed approximately three times faster than the corresponding acetate (33). The E value also increased considerably (Table 2.7). A maximum value, for this set of experiments, was achieved for the butyrate (34), E value = 35

Table 2.7 Effect of sustrate modification on both the rate of hydrolysis and the enantioselectivity.



sm *ca.* 60mg

sm #	R	Time/hrs	E
33	CH_3	72	14
34	$CH_3CH_2CH_2$	14	35
35	$CH_3(CH_2)_3CH_2$	14	27

The enantiomeric excesses and the subsequent E values were determined in the usual manner (see Section 2.8.1, Scheme 2.25). The butyrate experiment was repeated on a slightly larger scale (X2) to give (sm) (34) = 44% yield, 95%ee; (p) (29) = 56% yield, 82%ee; c = 0.537, E = 37. The (R)-(-)-residual starting material (34) was converted in two steps into the known (S)-(-)-aminoalcohol[104] (37) in 58% yield, see Section 2.10.

Two further experiments were conducted using the butyrate (34), employing the lipases from *Mucor* species and *Rhizopus javanicus*. These two lipases had previously been found to be the best enzymes for the resolution of the butyrate (26) (see Section 2.5, Table 2.3). It was found that both reactions proceeded with an E value of 10. However, the conversion was quite low after 14 hours i.e. lipase from *Rhizopus javanicus* gave (sm) (34) = 15%ee, (p) (29) = 82%ee; c = 0.15, E = 10. The lipase from *Mucor* species produced (sm) (34) = 10%ee, (p) (29) = 77%ee; c = 0.11, E = 10.

So with conditions maximised in the hydrolytic direction we turned our attention to using the lipase from *Pseudomonas fluorescens* in the reverse, esterification, direction.

2.9 LIPASE CATALYSED ESTERIFICATION

The esterification was attempted using two different systems. One employs potentially reversible conditions, whilst the other uses irreversible conditions.

2.9.1 POTENTIALLY REVERSIBLE CONDITIONS[22]

In these experiments the acyl donor was the free acid. The reaction solvent employed was isooctane and the enzyme, as usual, was used directly from the bottle (Table 2.8):

Table 2.8 Potentially reversible lipase catalysed esterification of chlorohydrin (29).

(29) *ca.* 50mg

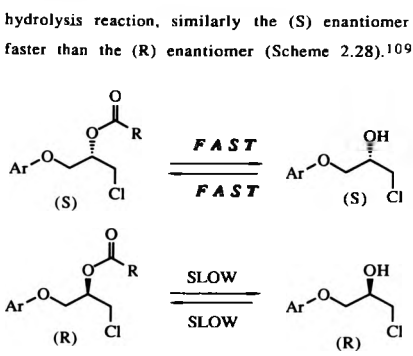
Biocatalyst:
lipase from *Pseudomonas fluorescens*,
isooctane,
55 °C.

R	E	c
CH_3	no reaction	
$CH_3CH_2CH_2$	104	0.12
$CH_3(CH2)_4CH_2$	74	0.05

Although the E value is very high, the rate of conversion is very slow, giving only 12% conversion after 24 hours. In another experiment butanoic anhydride was used as the acyl donor. However, non-enzymatic esterification occurred (as observed in a control experiment in which no enzyme was present).

The "sense" of the stereochemistry was preserved i.e. the (S) enantiomer was the faster reacting enantiomer in the

hydrolysis reaction, similarly the (S) enantiomer was esterified faster than the (R) enantiomer (Scheme 2.28).[109]



Scheme 2.28

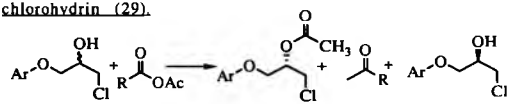
Because of the low rate of reaction, the conditions of the esterification were changed.

2.9.2 IRREVERSIBLE CONDITIONS[14]

The acyl donors in this case were either isopropenyl acetate[78] or vinyl acetate.[79] These have a major advantage over using the free acid as the acyl donor, in that concomitant with ester formation an enol is formed which rapidly tautomerises to the parent aldehyde or ketone. The position of enol to the aldehyde or ketone equilibrium lies firmly on the side of the aldehyde or ketone. Accordingly, the overall esterification reaction is essentially irreversible. Three experiments were set up. In one, vinyl acetate was used both as acyl donor and as the organic solvent. In the other two experiments, isopropenyl acetate was

used as the acyl donor. The organic solvent was either THF or CH_2Cl_2. These solvents were chosen merely on the grounds that the substrate (29) was soluble in both.[79] The lipase catalysed esterification was extremely clean in all three cases. The rate of conversion was greater when vinyl acetate was used, and slowest when CH_2Cl_2 was used as solvent. The experiments using vinyl acetate and THF were worked-up. The work-up was trivial. It involved simply filtering off the enzyme, and evaporation of the solvent, followed by flash chromatographic separation of product (33) and starting material (29). The E value was determined as usual. The results are given below (Table 2.9):

Table 2.9 Lipase catalysed irreversible resolution of chlorohydrin (29).

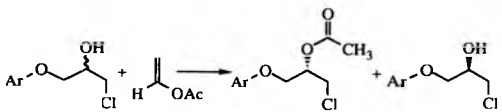


(29) (p), (S)-(33) (sm), (R)-(29)

Biocatalyst:
lipase from *Pseudomonas fluorescens*,
stir at room temperature.

Solvent	R	Time/hrs	c	E
THF	CH_3	96	0.22	130
CH_2Cl_2	CH_3	slow	n.d.	n.d.
Vinyl acetate	H	48	0.33	159

The reaction using vinyl acetate was scaled-up to 1g (20X). Since this reaction was the most enantioselective so far achieved, and the rate of conversion, although lower than in the hydrolytic reaction, was acceptable, especially given the dramatic increase in the E value (Scheme 2.29).



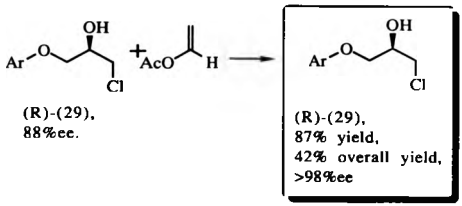
(1g) (S)-(33) 47% yield, >98%ee. (R)-(29) 47% yield, 88%ee.

Biocatalyst:
lipase from *Pseudomonas fluorescens*
65 hours, room temperature

c = 0.47
E = 502

Scheme 2.29

The apparent E value was 502. However, care should be taken when interpretating E values greater than 100, since very accurate determination of the %ee is required for a subsequent accurate calculation of the extent of conversion c.[70,81] What can be stated positively is that with E values greater than 100 the reaction is essentially completely enantioselective. The above reaction gave optically pure (S)-(33)-product. The residual starting material (29) (88%ee) was re-introduced to fresh enzyme. The contaminating 6% of (S)-(29) was successfully "siphoned off". The conventional work-up gave optically pure (R)-(29) (Scheme 2.30).



(R)-(29),
88%ee.

(R)-(29),
87% yield,
42% overall yield,
>98%ee

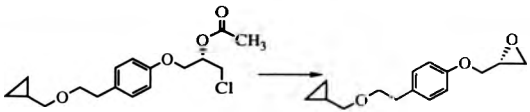
Biocatalyst:
lipase from *Pseudomonas fluorescens*
3 days, room temperature.

Scheme 2.30

Accordingly, with this result, an enzymatic approach to both enantiomers of optically pure β-blocker precursor (%ee > 98) had been achieved, with an overall yield of 89%. To complete the project successfully it was necessary to determine the absolute stereochemistry.

2.10 DETERMINATION OF THE ABSOLUTE STEREOCHEMISTRY

The optically pure (S)-(+)-(33), obtained by the lipase from *Pseudomonas fluorescens*-catalysed enantioselective esterification, was converted into the (S)-(-)-epoxide-(28) in 95% yield by treatment with potassium *tert*-butoxide (Scheme 2.31).

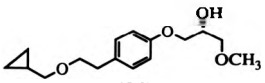


(S)-(+)-(33) (S)-(-)-(28)

Conditions:
potassium *tert*-butoxide,
tert-butanol, 60 °C, 30mins.

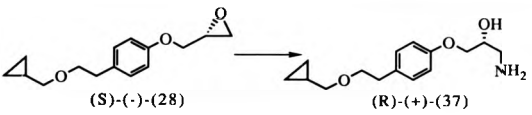
Scheme 2.31

When sodium methoxide had been used for the above conversion a significant amount of methyl ether (36) had been produced, either by nucleophilic ring opening of epoxide (28) or direct S_N2 displacement of the chloro group in the starting material (33):



(36)

The epoxide (S)-(-)-(28) was then converted into the known β-amino alcohol (R)-(+)-(37)[104] in 57% yield. The product was isolated by flash chromatography (Scheme 2.32).



(S)-(-)-(28) (R)-(+)-(37)

Conditions:
NH_3 (g), Methanol.

$[\alpha]_D = +18.06^o$

Literature $[\alpha]_D = +17.9^o$

Scheme 2.32

Similarly, the (R)-(-)-(34) enantiomer (95%ee) from the hydrolysis of racemic butyrate (34) (see Section 2.8.2) was converted to (S)-(-)-(37), *via* epoxide (R)-(+)-(28), in 58% overall yield $[\alpha]_D = -14.1^o$. By correlating the configuration of (37), with the ^1H n.m.r. (400 MHz) of the corresponding (R)-*O*-acetyl mandelic esters (32), the absolute configuration of all the samples of chlorohydrin (29) in this chapter have been assigned.

2.11 DETERMINATION OF ENANTIOMERIC EXCESS

The ee of chlorohydrin (29) was determined by synthesising the (R)-*O*-acetyl mandelic ester (32) (see Section 2.8.1)[106,107]. Examples of the ^1H n.m.r. (400 MHz) spectra of (2R,2'R,)-(32), >98%de; (2RS,2'R)-(32); and (2S,2'R)-(32), >98%de are given overleaf. Two regions of the spectra are reproduced. Figure 2.1 (a), (b), and (c) show the C-2' diastereomeric methine protons. Figure 2.2 (a), (b), and (c) show the diastereomeric acetyl methyl protons.

FIGURE 2.1 (a),(b), and (c) Methine region of the ^1H n.m.r. (400 MHz) spectra of (R)-(*O*)-Acetyl Mandelic ester (32) of 3-[4-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropanol:

(a) (2R,2'R)-(32), >98%de.

(b) (2SR,2'R)-(32),

(c) (2S,2'R)-(32), >98%de.

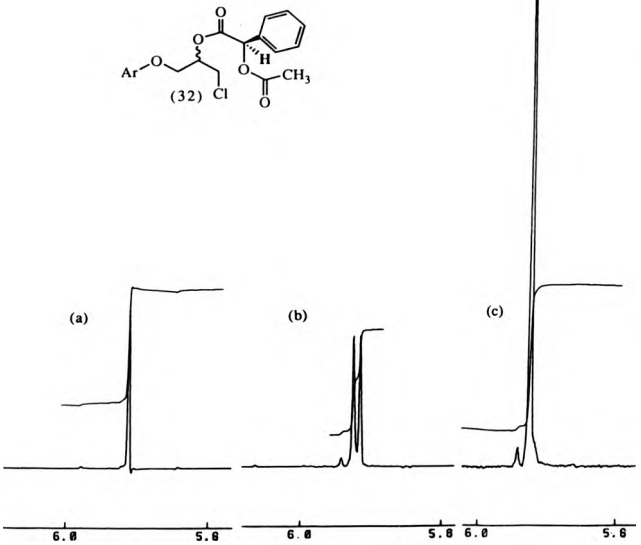
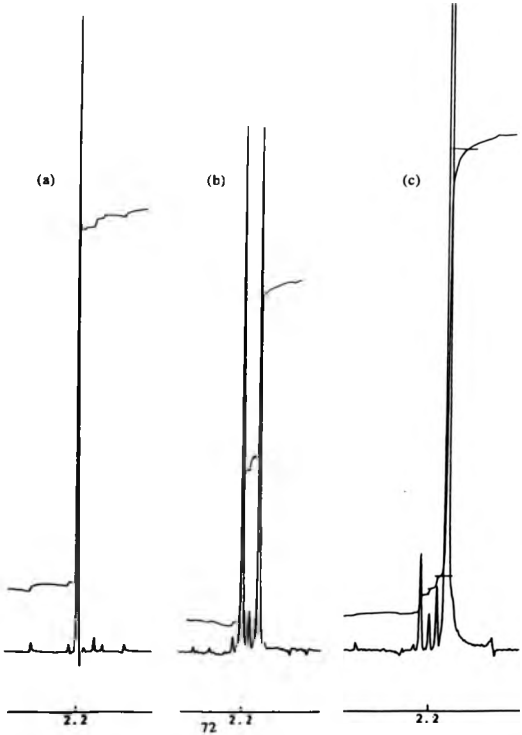


FIGURE 2.2 (a),(b), and (c) Acetate region of the ^1H n.m.r. (400 MHz) spectra of (R)-(*O*)-Acetyl Mandelic ester (32) of 3-[4-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropanol:

(a) (2R,2'R)-(32), >98%de.

(b) (2SR,2'R)-(32),

(c) (2S,2'R)-(32), >98%de.



2.12 SUMMARY

(1) The monooxygenase route to the β-blocker was unsuccessful. Racemic epichlorohydrin was synthesised in 5% yield using methane monoxygenase from *Methylococcus capsulatus* (Bath).[98]

(2) Microbiological reduction of α-chloroketone (21) proceeded in low yield, with moderate to good ee. Acetate hydrolysis was also observed.

(3) Enzymatic hydrolysis of racemic butyrate (26) produced one enantiomer in >98%ee, 36%yield. However the hydrolysis was not completely regio- and chemioselective. Competing acetate hydrolysis was observed.

(4) Microbiological reduction of the cyclopropylmethyl protected precursor (30) proceeded with only moderate ee and yield. The results were complicated by the starting material sensitivity.

(5) Hydrolytic enzymatic resolution of racemic ester (33) gave good results. From an initial screen of enzymes, the lipase from *Pseudomonas fluorescens* was selected for further study (E = 14).

(6) Substrate modification[71] resulted in an increase in both the rate of hydrolysis and the enantioselectivity. The butyrate ester (34) was selected as the best substrate (E = 37).

(7) In the esterification direction, the "sense" of the chirality of the enzymes stereoselectivity was preserved.[109] The (S)-enantiomer is both hydrolysed and esterified faster than its enantiopode.

(8) Using potentially reversible conditions[22] the E value was >100. However, the rate of esterification was very slow.

(9) The use of irreversible conditions[78,79,80] increased the enantioselectivity. E>100, the rate of esterification was tolerable.

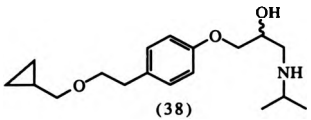
(10) The use of vinyl acetate[79] led to both enantiomers in an optically pure state (%ee > 98), in 89% overall yield.

(11) The stereochemistry was proved by conversion of the enzymatic product to the known β-aminoalcohol (37),[104] *via* the epoxide intermediate (28).

(12) The β-blocker (38), was synthesised in racemic form, by two methods:

(A) Regioselective opening of epoxide (28) with isopropylamine (87%).[111,112]

(B) Direct displacement of the chloro group of the chlorohydrin (29) by isopropylamine (56%).



(38)

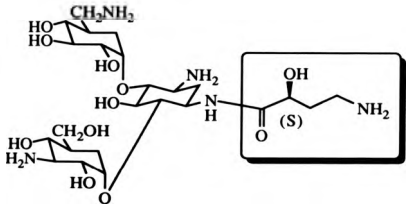
So a formal synthesis of both enantiomers of the desired target molecule (38) has been demonstrated. The synthesis used currently available enzyme technology, coupled with conventional chemistry.

CHAPTER THREE

The stereospecific production of optically enriched (2S)-4-amino-2-hydroxybutanoic acid analogue.

3.1 INTRODUCTION

(L)-(2S)-4-amino-2-hydroxybutanoic acid (L-AHBA) (40) is an unusual, naturally occurring amino acid. It is a component of the natural product kanamycin.[116] Structural modification of kanamycin lead to development of amikacin (41) which is a broad-spectrum antimicrobial antibiotic:

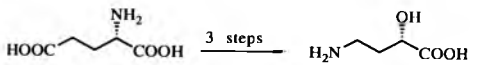


(41)

L-AHAB is one of the most potent inhibitors of the neurotransmitter γ-aminobutanoic acid known to man.[117] AHAB also exhibits anti-cancer activity.[118]

Various chemical syntheses of racemic and L-AHAB (40) have been reported.[116] Most routes to optically active L-AHAB either start from amino acids (e.g. Scheme 3.1), or involve a chemical resolution step. A microbiological resolution route has also been

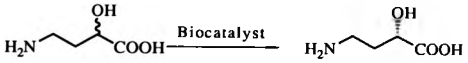
reported, in this case the D-enantiomer is preferentially metabolised (Scheme 3.2).[116]



(S)-(L)-(+)-glutamic acid

(S)-(L)-AHBA (40)
40% overall yield

Scheme 3.1



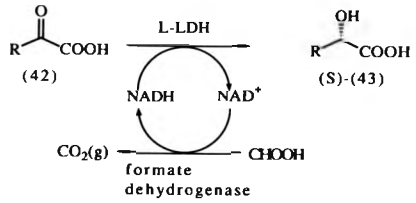
(R,S)-(40)

(S)-(L)-(40)
41% yield

Biocatalyst:
Acetobacter sp. ATCC 21780
3 days, 28 °C

Scheme 3.2

Whitesides and Kim have shown[119] that the inexpensive enzyme L-lactate dehydrogenase, L-LDH (E.C. 1.1.1.27) (US$10 per 10 units) reduces 2-oxo acids (42) in excellent yields and stereospecificity, to produce the (S)-alcohol-(43), in fairly large preparative scales (e.g. 6g) (Scheme 3.3).



Scheme 3.3

However, as the R group becomes larger the k_{cat}/K_m dramatically falls off. No examples were quoted with a nitrogen substituent in the R group.[119]

Recently there appeared a literature report that enantiomerically pure β-keto ester (44) could be stereospecifically reduced to the corresponding alcohol (45).[120] Chemical hydrogenation of the phenyl ring produced a protected form of an anologue of statine, ((3S,4S)-4-amino-3-hydroxy-6-methylheptanoic acid), a key component in several renin inhibitors. The workers found that baker's yeast reduction proceeded with only moderate stereospecificity (60%ee, 30% yield). A screen of 75 yeasts was set up. Only five yeasts reduced (44) completely. *Hansenula anomala* was selected; it produced the β-hydroxy ester (45) in 92%ee (Scheme 3.4). The reaction was successfully scaled-up to 50g. Two recrystallisations of (45) gave optically pure product in approximately 70% yield.

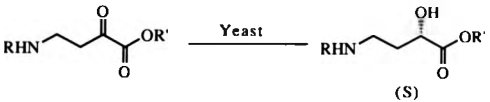
(44) (45) 92% ee

Scheme 3.4

3.2 AIMS

We aimed to reduce 2-oxo acid analogues of 4-amino-2-oxo-butanoic acid using yeasts to produce the (2S)-alcohol enantiospecifically, followed by subsequent chemically conversion to the desired alcohol ((2S)-4-amino-2-hydroxybutanoic acid), or its protected analogues. The strategy can be summed up as follows (Schemes 3.5 and 3.6):

(A) Enantiospecific yeast reduction of a linear 2-oxo starting material (Scheme 3.5):

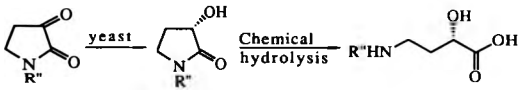


(S)

R = H or Protecting group (PG).
R' = H or PG.

Scheme 3.5

(B) Enantiospecific yeast reduction of a cyclic 2-oxo-lactam (Scheme 3.6):



R" = H or PG.

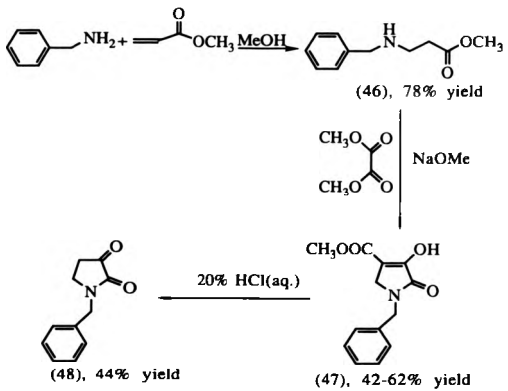
Scheme 3.6

The example of the production of a statine derivative in (Section 3.1) encouraged us to screen as wide a variety of yeasts as possible, to enable us to have a better chance of finding a biocatalyst with the desired enantiospecific reducing properties.

3.3 SYNTHESIS OF THE TERTIARY LACTAM (48)

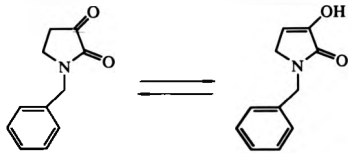
The first target molecule (48) was synthesised (Scheme 3.7) following the route of Southwick and Crouch.[121] Michael addition of benzylamine to methylacrylate produced methyl-β-benzylaminopropionate (46). The ester (46) was treated with dimethyl oxalate under basic conditions to produce 4-carbomethoxy-1-benzyl-2,3-dioxopyrrolidine (47) *via* a Dieckmann-type condensation and ring closure. Both reactions worked well to produce 0.6 mole of recrystallised (47) as beautiful, shiny, white crystals. The next step involved the acid catalysed hydrolysis and decarboxylation of (47) to yield 1-benzylpyrrolidine-2,3-dione (48). This step proved problematical. The literature method was not reproducible.[121]

However, a literature search revealed that several other authors had encountered problems with this step.[122,123,124] The isolation and purification of ester (48) was solved following the method of Baldwin *et al.*[123]



Scheme 3.7

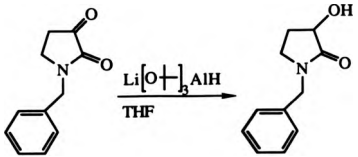
Compound (48) is unstable to silica gel chromatography. The ^1H n.m.r. (200 MHz) spectrum shows that the compound exists, in $CDCl_3$ solution, completely as the tautomeric keto form. However, the compound produces two u.v. active spots on t.l.c. suggesting that the compound enolises under polar conditions (Scheme 3.8).



(48) (48)

Scheme 3.8

This can be compared to compound (47) which exists completely as the enol form, in $CDCl_3$ solution, as indicated by ^1H n.m.r. This tendency of compound (48) to enolise, coupled with its inherent instability led to problems when attempting to analyse the microbiological reduction of (48). A standard reduced alcohol was synthesised (Scheme 3.9). Reduction of (48) by sodium borohydride gave alcohol (49) but only in 27% yield after flash chromatography. The yield was improved by reducing (48) with lithium tri-*tert*-butoxyaluminohydride (Scheme 3.8).[125]



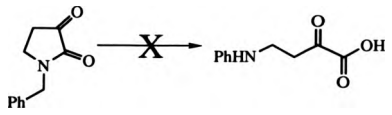
(48) (49)

Scheme 3.9

This reaction was not accurately reproducible on a large scale. Variable yields were obtained (19-63 %yield).

Yeast reductions of (48) were attempted by the laboratory technician (David Anderson). A screen of ten yeasts was set up. Reduction was observed, by t.l.c., but were incomplete. The reaction mixtures were not isolated.

The tertiary lactams (48), (49) and (50) could not be hydrolysed to the open chain amino acids (51), (52) and (53) under any conditions (see Schemes 3.10, 3.12 and 3.13).



(48) (51)

Scheme 3.10

Conditions:

(1) NaOMe, methanol, reflux.

(2) KOH (1.1eq.), MeOH, reflux.

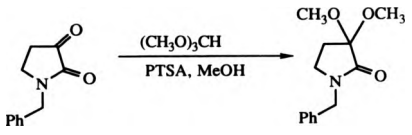
(3) KOH (10eq.), MeOH, reflux.

(4) 20% v/v HCl (aq.), reflux.

(5) Potassium *tert*-butoxide under naked anion conditions.[126]

The reaction either produced a non-characterised mess, or starting material was re-isolated. Perhaps the enolisation of the ketone (48) (see Scheme 3.8) was hampering the hydrolysis. To

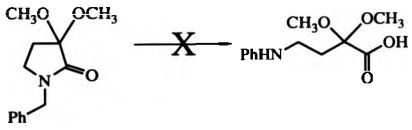
this end ketal (50) was synthesised following the method of Sunberg *et al.* (Scheme 3.11).[124]



(48) (50), 79% yield

Scheme 3.11

However it was not possible to hydrolyse (50) under basic conditions (Scheme 3.12).



(50) (52)

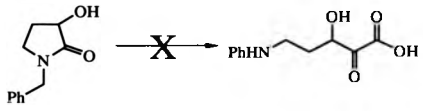
Scheme 3.12

Conditions:

(1) KOH, ethanol, reflux.

(2) Potassium *tert*-butoxide under naked anion conditions.[126]

Similarly the alcohol (49) was not hydrolysed under basic conditions (Scheme 3.13).



(49) (53)

Scheme 3.13

Conditions:

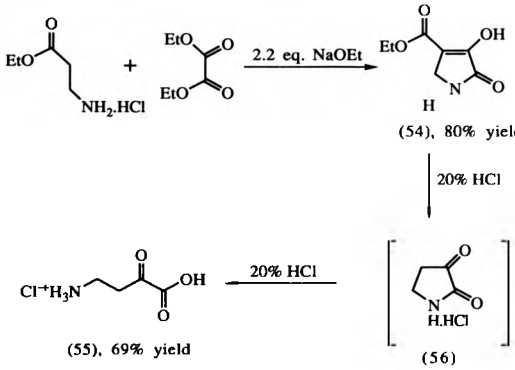
(1) NaOEt, EtOH, reflux.

(2) KOH (7eq.), EtOH, reflux.

Tertiary amides are hard to hydrolyse.[126] In this case the tertiary lactams failed to give the desired open chain amino acid, under the above conditions. Due to these insurmountable problems it was decided to synthesise the secondary 2-oxolactam followed by hydrolysis and selective protection of both terminals of the amino acid.

3.4 SYNTHESIS OF THE DIPROTECTED 2-OXO AMINO ACID (60).

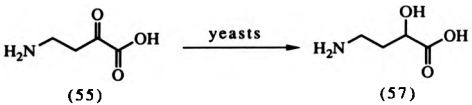
In a modification of the Southwick and Crouch procedure,[121] 4-carboxyethoxypyrrolidine-2,3-dione (54) was prepared by reacting β-alanine ethyl ester as its HCl salt with diethyl oxalate and 2.2 mole equivalents of sodium ethoxide. The base neutralises the β-alanine ethyl ester HCl salt and mediates a Dieckmann-type condensation followed by ring closure. Acidic work-up yields lactam (54). This was decarboxylated and hydrolysed directly to 4-amino-2-oxo-butanoic acid as its HCl salt (55) (Scheme 3.14).



Scheme 3.14

Von Dobeneck originally synthesised the amino acid (55) but assigned it the secondary lactam structure (56) as its hydrochloride monohydrate salt.[122] The correct structure of (55) was elucidated by Sunberg *et al.* in 1986.[124]

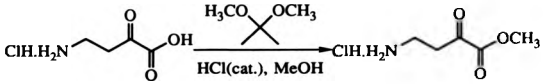
Yeast reduction on the water soluble neutralised 2-oxo acid (55) was attempted to the desired alcohol (57)[127] (Scheme 3.15).



Scheme 3.15

purified. Ten yeasts were screened for the reduction of 2-oxo acid (55). The reduction appeared to be slow (>70 hours). The reactions were difficult to monitor with confidence, product isolation of the water soluble alcohol (57) would have been laborious.

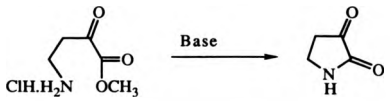
Therefore it was decided to selectively di-protect the amino acid (55). This proved to be non-trivial. Firstly eight attempts to protect the nitrogen terminal of the free amino acid were unsuccessful, apart from one reaction which produced a t-BOC derivative in 36% yield. Protection of the carboxylic acid function of (55) was more successful, which using the general method of Rachele,[128] produced the methyl ester (58) (Scheme 3.16).



(55) (58), 61% yield

Scheme 3.16

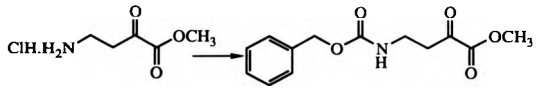
Protection of the amine group of (58) proved to be difficult. One problem might have been that when the free amine group is exposed an intramolecular ring closure to form the lactam (59) could occur (Scheme 3.17).



(58) (59)

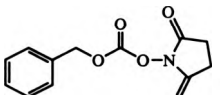
Scheme 3.17

Five different sets of conditions failed to protect (58). However using an activated ester (61) of the benzyloxycarbonyl, CBZ or Z, group, and exactly one equivalent of base, an acceptable yield of (60) was obtained (Scheme 3.18).[129]



(58) (60), 50-84% yield.

Conditions:



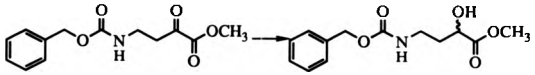
(61)

. Triethylamine (1 eq.), CH_2Cl_2, O °C to RT.

Scheme 3.18

Isolation of (60) by flash chromatography always resulted with some co-elution of the derivatising agent (61). However, by carefully controlling the reaction conditions, samples of (60) were obtainable contaminated with only 2% of the derivatising agent (61). Access to an organic soluble 2-oxo acid derivative (60) was now in hand. Additionally, compound (60) contained an u.v. marker, permitting easy monitoring of yeast reductions.

A reduced racemic standard (62) was synthesised by sodium borohydride reduction of (60) (Scheme 3.19).[130]

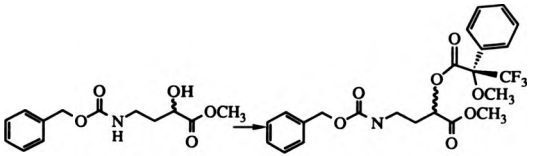


(60) (62), 52% yield.

Conditions:
$NaBH_4$, THF, -13 °C, 20 minutes.

Scheme 3.19

Several methods for calculation of the ee of alcohol (62) were tried. The method selected involved the synthesis of the corresponding (R)-Mosher's ester (63) (Scheme 3.20).[103]



(62) (63)

Conditions:
(S)-(+)-MTPA-Cl, pyridine, CCl_4.

Scheme 3.20

A ^1H n.m.r. (200 MHz) spectrum of (63) revealed beautifully separated resonances for both sets of diastereomeric methoxyl

protons. One set resonated at δ = 3.39 and 3.47 p.p.m., whilst the other set resonated at δ = 3.55 and 3.76 p.p.m. Thus an excellent method for the determination of the %ee of alcohol (62) had been established. The technique has an internal standard which minimises errors, which might arise because of underlying impurity peaks (*c.f.* Section 2.11). Examples of ^{1}H n.m.r. spectra are given in Section 3.7. Determination of the absolute stereochemistry is established in Section 3.6.

3.5 YEAST REDUCTIONS OF COMPOUND (60)

Initially baker's yeast (Red Star) obtained from a local supermarket (Woodmans) was used as the biocatalyst. The reduction of ketone (60) proceeded cleanly to yield the desired alcohol (62). When the reaction was complete the reaction mixture was centrifuged. The aqueous supernatant was extracted with organic solvent, and the pooled organic solvents were dried and evaporated. The alcohol (62) was purified by preparative layer chromatography (P.L.C.). The corresponding Mosher's ester (63)[103] was synthesised as above and purified after work-up by P.L.C. Using baker's yeast, (S)-(62) was obtained in 50% yield, 77%ee. Although the yield was reasonable the ee was only moderate to good. So, in the pursuit of a more efficient biocatalyst, a yeast reduction screen was set up. The yeasts were selected from the laboratory culture collection. Pure strains were transfered to sterilised Vogel's media (10ml) in a conical flask, and shaken vigorously at 37 ^{o}C, on a rotatory shaker. When the broth was thick with multiplying yeasts (usually after twenty-four hours) an aliquot

(1ml) was transferred to fresh Vogel's media (10ml). The contents of this conical flask was then incubated for a further twenty-four hours before the substrate was administered. In all the cases described below, the reduction was carried out at the relatively strong substrate concentration of 3mg/ml. The solid substrate (60) (30mg) was dissolved in DMSO (0.3ml, final concentration of DMSO = 3%v/v) and added to the broth. The reduction was followed periodically by t.l.c. When the reaction was adjudged complete, the product (62) was isolated and converted into its corresponding Mosher's ester (63),[103] following the procedure outlined above. In all, twenty yeasts were cultivated and screened for their ability to reduce the starting ketone (60). The ketone was an extremely good substrate. In twelve cases the reduction was complete after approximately one day. The results for these twelve yeasts are given in the following table (Table 3.1).

Table 3.1 Yeast reductions of the 2-oxo-ester (60).

(60), 3mg/ml (S)-(+)-(62)

Yeast name	Yeast #	Time /hrs	%yield	%ee	R/S
Saccharomyces carlsbergensis	ATCC 2345	28	40	88	S
Acinetobacter lwoffi	17946	28	42	72	S
S. cerevisiae var.ellipsoides	ATCC 10607	28	38	71	S
Candida tropicalis	ATCC 20336	28	25	14	R
?	134	28	13	40	S
Saccharomyces cerevisiae (ale)	NCYC 1003	21	49	57	S
Saccharomyces ellipsoides	1016	21	60	83	S
Yarrowia lipolytica	NCYC 925	21	56	73	S
Candida lipolytica	925A	21	29	73	S
Saccharomyces cerevisiae	NCYC 114	21	25	17	S
Candida guilliermondii	ATCC 9058	21	9	78	S
Beer yeast	"Edna"	21	54	88	S

From Table 3.1 it can be seen that:

(1) The yeasts preferentially produce the (S)-alcohol (62). The ee varies from 17-88%.

(2) The yield varies from 9-56%.

(3) In only one case is the (R)-alcohol (62) produced, and then in only 14%ee.

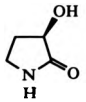
(4) The best yeasts are *Saccharomyces carlsbergensis* ATCC 2345 (88%ee, 40% yield) and a laboratory strain "Edna" (88%ee, 54% yield).

In summary, the most enantiospecific reduction produced alcohol (S)-(+)-(62) in 88%ee. This represents an enantiomer

ratio of 16 : 1. The next task was to elucidate the stereochemistry.

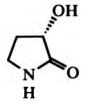
3.6 DETERMINATION OF THE ABSOLUTE CONFIGURATION

Optically enriched methyl-4-(benzyloxycarbonyloxy)-4-amino-2-hydroxybutanoate (62) (77%ee), obtained by the baker's yeast reduction of the corresponding ketone was used for the determination of the absolute configuration. It was noted in the literature that the absolute configuration of both enantiomers of 3-hydroxy-2-pyrrolidone (64) had been established[131] (Scheme 3.21).



(R)-(+)-(64)

$[\alpha]_D = +121.9^o$

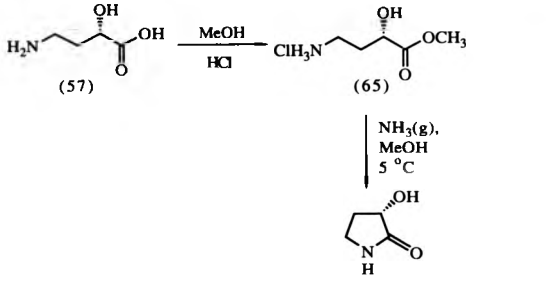


(S)-(-)-(64)

$[\alpha]_D = -113^o$

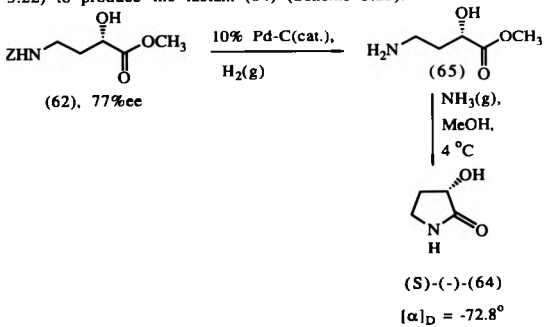
Scheme 3.21

The (S)-(-)-enantiomer of hydroxylactam (64) had been made from (S)-4-amino-2-hydroxybutanoic acid (57) *via* esterification to the methyl ester (65), followed by neutralisation and ammonia catalysed lactamisation (Scheme 3.22).[131,132,133]



Scheme 3.22

Thus, the alcohol product (62) was converted into lactam (64). This was achieved by removing the Z-protecting group to liberate the free amine, which was lactamised (as in Scheme 3.22) to produce the lactam (64) (Scheme 3.23).



Scheme 3.23

Thus, the absolute configuration of the lactam (64) derived from the yeast product (62) was determined to be (S). The absolute configuration of the yeast product from baker's yeast can then be correlated to the absolute configuration of the alcohols (62) derived from the other yeasts by simple comparison of the proton n.m.r. spectra of their corresponding Mosher's esters (63).[103]

It is noteworthy that the optical rotation of the yeast product (62) is very small at the sodium D line $[\alpha]_D$ = + 1.4 °. The specific rotation was measured for a series of wavelengths: λ = 589, 578, 546, 436 and 365 nm. The rotation was greatest at λ = 365 nm, $[\alpha]_{365}$ = +14.5 °. However, at this wavelength the mercury lamp which had been used to generate the monochromatic light was not very stable. Therefore, in studying the stereospecificity of the reduction of (60) to (62), measurement of the specific rotation is relatively worthless.

3.7 DETERMINATION OF THE ENANTIOMERIC EXCESS

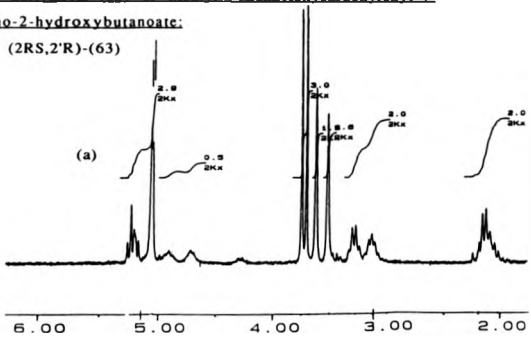
The %ee was determined by synthesising the (R)-Mosher's ester (63) as described in Section 3.4, Scheme 3.20.[103] The purified Mosher's ester was then analysed by ^{1}H n.m.r. (200 MHz) The two sets of methoxy resonances are diagnostic for calculating the ee. One set of methoxy signals resonate as singlets at δ = 3.39 and 3.47 p.p.m. The resonance at δ = 3.39 p.p.m. (the upfield singlet) corresponds to the (2S,2'R(MPTA))-(63)-diastereomer. The other set of methoxy resonances appear at δ = 3.55 and 3.76 p.p.m. Again the upfield

resonance is due to the (2S,2'R(MPTA))-(63)-diastereomer, which can be extrapolated back to the (S)-(62)-enantiomer.

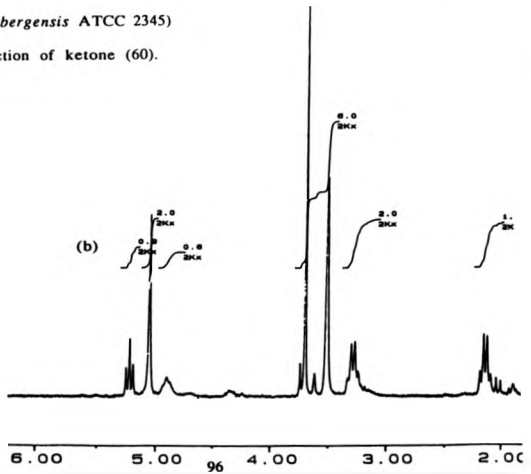
Overleaf two examples of the ^1H n.m.r. (200 MHz) spectra are reproduced. Firstly, (63) synthesised from racemic (62) is given (Figure 3.1 (a)). Then, secondly, (63) derived from 88%ee (S)-(62) (the product when *Saccharomyces carlsbergensis* ATCC 2345 was used as the biocatalyst in the reduction of (60) (Figures 3.1 (b)).

FIGURE 3.1 (a) and (b) ^1H n.m.r. (200 MHz) spectra of Mosher's ester derivative (63) of Methyl-4-(Benzyloxycarbonyloxy)-4-amino-2-hydroxybutanoate:

(a) (2RS,2'R)-(63)



(b) (2S,2'R)-(63), 88%de obtained *via* the yeast (*Saccharomyces carlsbergensis* ATCC 2345) reduction of ketone (60).



3.8 SUMMARY

(1) The tertiary lactams (48), (49) and (50) were synthesised. They could not be hydrolysed to the open chain amino acids.[126]

(2) Synthesis of 2-oxo secondary lactam led directly to 4-amino-2-oxo-butanoic acid, HCl salt (55).[124]

(3) Yeast reduction of (55) was slow. The water soluble product was not isolated.

(4) Selective protection of both the carboxylic acid and amine functionalities was achieved, to produce (60).

(5) Yeast reduction of (60) was successful. Of the twenty yeast screened, twelve yeasts completely reduced (60), in about one day.

(6) The enantiomeric excess varied from 17-88%.

(7) The yields varied from 9-56%.

(8) The stereochemistry of the alcohol (62) was elucidated to be (S)-(+)-(62), in 93% of the yeasts screened.

(9) The best yeasts were *Saccharomyces carlsbergensis* ATCC 2345 (88%ee, 40% yield) and a laboratory beer yeast strain "Edna" (88%ee, 54% yield).

So, in this chapter a stereospecific route to a protected form of (2S)-4-amino-2-hydroxybutanoic acid (88%ee) has been demonstrated. Additionally a route to (3S)-3-hydroxy-2-pyrrolidone (64) (77%ee) has been detailed. The yeast reduction of the 2-oxo acid compound (60) gave, as expected, the 2S-hydroxy acid compound (62).

CHAPTER FOUR

Stereospecific yeast reduction of 4-(phenylthio)butan-2-one and related compounds.

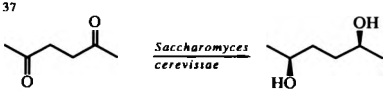
4.1 INTRODUCTION

The reduction of prochiral methyl ketones by baker's yeast (*Saccharomyces cerevisiae*) almost invariably produces the corresponding (S)-alcohol.[14,18,44] Hydride addition occurs to the *re*-face of prochiral methyl ketones, that is Prelog's rule is observed[46] (see Section 1.2.2). Some examples are given in Scheme 4.1.[46,137-145]

A.[46]

(S)

B.[137]



(S,S), 50% yield, 96%ee.

C.[138]

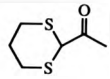
(S)-(69), ≥97%ee

D.[139]

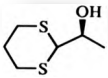
(S), 98% yield, 100%ee.

Scheme 4.1

E.[140]

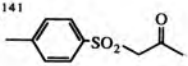


Saccharomyces cerevisiae

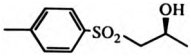


(S), 84% yield, >96%ee.

F.[141]

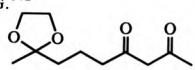


Saccharomyces cerevisiae

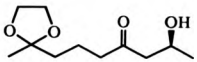


(S), no yield quoted, 97%ee.

G.[142]



Saccharomyces cerevisiae



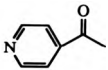
(S), 65% yield, 97%ee.

H.[143]

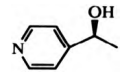
Saccharomyces cerevisiae

R > CH3,
(seven examples) (S), 41-94% yield,45 to >96%ee.

I.[144]

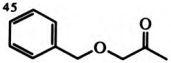


Saccharomyces cerevisiae

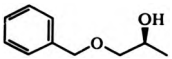


(S), 90% yield, 96%ee.

J.[145]

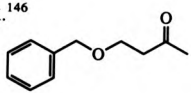


Saccharomyces cerevisiae

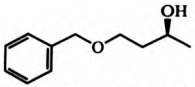


(S), 76% yield, 90%ee.

K.[146]



Saccharomyces cerevisiae

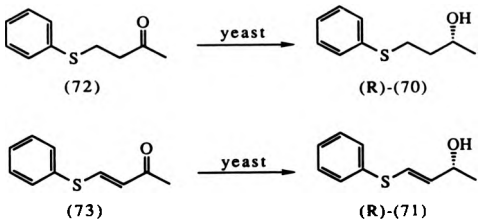


(S), 52% yield, 95%ee.

Scheme 4.1 (continued).

4.2 AIMS

This section of the thesis is concerned with the stereospecific synthesis of two chirons (70) and (71). In particular, we aimed to try to produce the (R)-enantiomers of alcohols (70) and (71). We envisaged that this might by achieved by the stereospecific yeast-mediated reduction of the corresponding ketones (72) and (73) (Scheme 4.2). Prelog's rule predicts that methyl ketones would be reduced by *Saccharomyces cerevisiae* to yield (S)-alcohols (Scheme 4.1).[46] We hoped to screen a large number of yeasts in the aim of finding a microorganism with an opposite stereochemical preference compared to that of *Saccharomyces cerevisiae*.

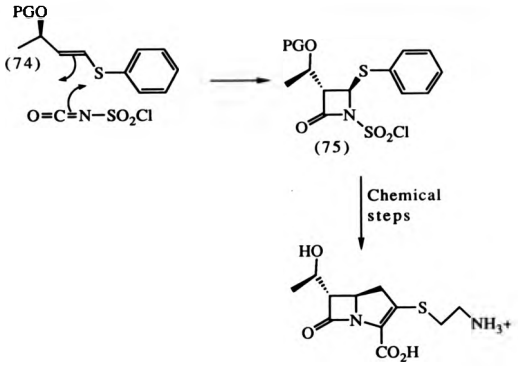


(72) (R)-(70)

(73) (R)-(71)

Scheme 4.2

If (2R,3E)-4-(phenylthio)but-3-en-2-ol (71) is protected as its silyl ether (74) it can then be considered as part of a key intermediate (75) in the synthesis of penems and carbapenems (e.g. 76)[146,85] (Scheme 4.3) (*c.f.* with the synthesis of thienamycin (76)[146] and related compounds[147,148]).

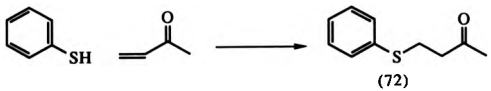


Thienamycin (76)

Scheme 4.3

4.3 SYNTHESIS OF METHYL KETONES (72) AND (73)

4-(Phenylthio)butan-2-one (72) was synthesised (Scheme 4.4) by the Michael addition of thiophenol to methyl vinyl ketone. The reaction was catalysed by tetrabutylammonium fluoride (TBAF).[149]



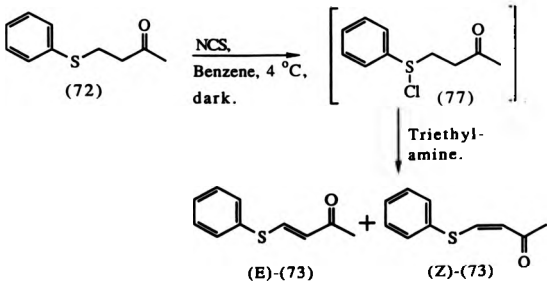
(72)

Conditions:
Tetrabutylammonium fluoride (cat.),
THF,
1 - 25 minutes.

Scheme 4.4

The amount of TBAF used was varied. It was found that as little as 0.005 mole TBAF could catalyse the reaction on a one mole scale. When the reaction was judged complete (by t.l.c.), a slight excess of methyl vinyl ketone was added to remove any traces of thiophenol which may have been present. The ketone (72) was isolated by evaporation of the solvent, followed by flash chromatography.

The saturated ketone was then converted into the corresponding αβ-unsaturated ketone, (3E)-4-(phenylthio)but-3-en-2-one (73) (Scheme 4.5). The reaction proceeds *via* the intermediate, 4-chloro-4-(phenylthio)butan-2-one (77), in a stereospecific reaction. This literature method of Bakuzis and Bakuzis[150] was performed in three different organic media: carbon tetrachloride, acid washed carbon tetrachloride[151] and benzene. Dry benzene was found to be the best solvent.



(72) (77) (E)-(73) (Z)-(73)

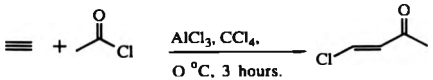
E : Z ; 10 :1

NCS = N-chlorosuccinimide

Scheme 4.5

The reaction was monitored by ^1H n.m.r. (200 MHz). When all the chlorosulphide (77) had reacted the ratio of E : Z αβ-unsaturated ketones (73) was 10 : 1. Mild acid treatment in the work-up resulted in isomerisation of the double bond. Flash chromatographic separation of the E and Z isomers was possible to yield pure E and Z in a ratio of 3 : 1. It was subsequently established by a co-worker (Gu-Ming Guo) that if the acid treatment was omitted the ratio of E : Z remained at 10 : 1.

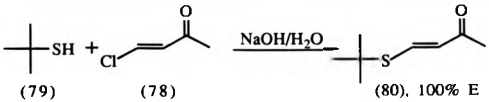
An alternative route to (3E)-4-(phenythio)but-3-en-2-one (73) was attempted but was not so successful.
(3E)-4-Chlorobut-3-en-2-one (78) was synthesised by the Lewis acid mediated addition of acetylene to acetyl chloride (Scheme 4.6).[152,153]



(78), 23% yield.

Scheme 4.6

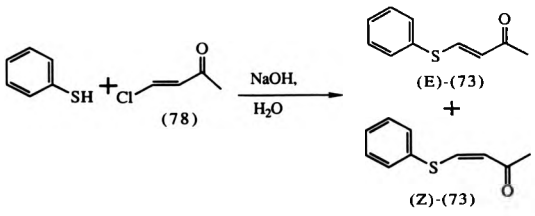
A report in the literature stated that the addition of *tert*-butylmercaptan (2-methyl-2-propanethiol) (79) to (3E)-4-chlorobut-3-en-2-one (78), under basic conditions, produced exclusively (3E)-4-(*tert*-butylthio)but-3-en-2-one (80) (Scheme 4.7).[154]



(79) (78) (80), 100% E

Scheme 4.7

Accordingly, thiophenol was added to (3E)-4-chlorobut-3-en-2-one (78), under the same conditions. However this produced a mixture of E : Z αβ-unsaturated ketone isomers (73) in the ratio 2.5 : 1 , by ^1H n.m.r. (200 MHz) (Scheme 4.8).

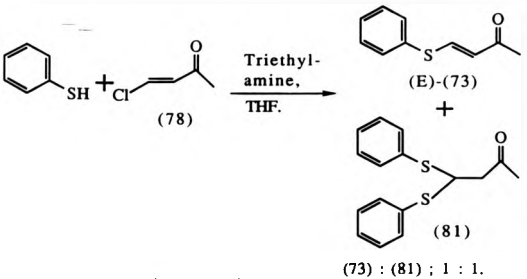


(78)

E : Z ; 2.5 :1

Scheme 4.8

Changing to a non-hydroxylic solvent gave a 1 : 1 mixture of E-(73) isomer and diadduct (81), by ^1H n.m.r. (Scheme 4.9).



Scheme 4.9

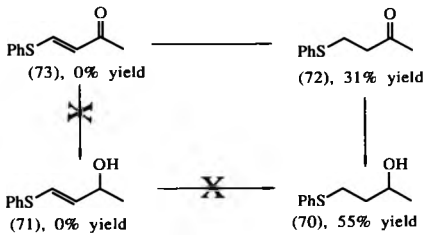
Accordingly, the modified route of Bakuzis and Bakuzis[150] was followed (Scheme 4.5) to produce (3E)-4-(phenylthio)but-3-en-2-one (73).

4.4 YEAST REDUCTION OF KETONE (73)

Nine yeasts were screened for their ability to reduce ketone (73) to allylic alcohol (71) (see Scheme 4.2). The yeast reactions were monitored by t.l.c. It was observed that ketone (73) was reduced. However, it was apparent that the olefinic double bond of ketone (73) was reduced to produce the saturated ketone (72). Subsequently, ketone (72) was further reduced to 4-(phenylthio)butan-2-ol (70). (Scheme 4.10).

To confirm the t.l.c. data the reduction of (73) catalysed by the yeast *Candida lipolytica* 5699 was worked-up. The products were isolated and purified by flash chromatography. The mixture yielded 4-(phenylthio)butan-2-one (72) (31% yield) and

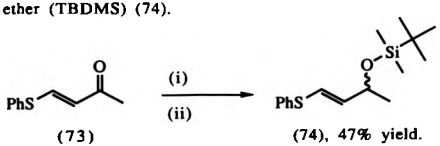
4-(phenylthio)butan-2-ol (70) (55% yield). No allylic alcohol (71) was isolated (Scheme 4.10).



Conditions:
Candida lipolytica 5699,
Vogels's medium,
30 °C, 6 days.

Scheme 4.10

A racemic standard of the desired alcohol (2R,S)-(3E)-4-(phenylthio)butan-2-ol (71) was synthesised by sodium borohydride reduction of the ketone (73). However, the allylic alcohol (71) was unstable at room temperature. It was stabilised by protecting the alcohol as its *tert*-butyldimethylsilyl ether (TBDMS) (74).



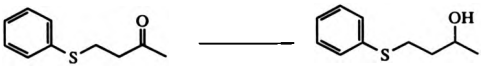
Conditions:
(i) $NaBH_4$, MeOH, -6 °C, 20 minutes.
(ii) TBDMS-Cl, imidazole, DMF.

Scheme 4.11

Since yeast reduction of αβ-unsaturated ketone (73) produced the saturated compounds (72) and (70), coupled with the observation that the allylic alcohol (71) is unstable, prompted us to concentrate on the yeast reduction of 4-(phenylthio)butan-2-one (72).

4.5.1 BAKER'S YEAST REDUCTION OF KETONE (72)

Baker's yeast (Red Star) reduction of 4-(phenylthio)butan-2-one (72) was extremely slow. It took five days for the reaction to go to completion, even at the relatively low substrate concentration of 1.13g/l. Isolation of the alcohol (70) by centrifugation, extraction and flash chromatography gave alcohol (70) in 54% yield as a slightly yellow, clear liquid (Scheme 4.12).



(72)

(+)-(70), 54 %yield

$[\alpha]_D = +33.5^{\circ}$ (c 0.94, $CHCl_3$)

Conditions:
Baker's yeast (Red Star) (10g),
sucrose (10g),
water (100ml),
room temperature, 5 days.

Scheme 4.12

4.5.2 ABSOLUTE CONFIGURATION OF THE ALCOHOL (70)

Fujisawa *et. al.* reported that baker's yeast reduction of 4-(phenylthio)-butan-2,3-dione (82) gave predominantly *anti*-(2S,3S)-4-(phenylthio)-2,3-butanediol (83) in 52% yield,

72%de. The %ee was not quoted (Scheme 4.13). Diol (83) was recrystallised to optical purity (83).[155]

(82) (2S,3S)-(83)

Scheme 4.13

To determine the stereochemistry Fujisawa *et. al.* stated that they monotosylated diol (83). Subsequent reduction (displacement?) with $LiAlH_4$ gave alcohols (70) and (84) (Scheme 4.14).[155]

(S)-(-)-(70) (S)-(+)-(84)

$[\alpha]_D = -28.3$ °

Scheme 4.14

To the (-)-enantiomer of alcohol (70) the (S)-configuration was assigned. This was established by comparison of the sign of rotation of the (R)-alcohol-(70) which was obtained after a thirteen step synthesis based loosely on the work of Mori *et. al.*[156]

Thus, if Fujisawa *et. al.*'s stereochemical assignment of alcohol (70) is correct, baker's yeast reduction of ketone (72) produces (R)-(+)-alcohol-(70) (see Scheme 4.12). This observation is a

direct contradiction of Prelog's law.[46] As stated in the introduction (see Section 4.1) prochiral methyl ketones are reduced by *Saccharomyces cerevisiae* to give the corresponding (S)-alcohols (see Scheme 4.1). Therefore we postulated that although Fujisawa *et. al.* had assigned the stereochemistry of the diol (83) (see Scheme 4.13) correctly, they had in some way made an error in their method for assigning the absolute stereochemistry of the isolated component alcohol (70). (This was, indeed, confirmed in a personal communication between Professors C. J. Sih and T. Fujisawa[157]).

Accordingly, we concluded that the product (70) from the baker's yeast reduction of ketone (72) to be (S)-(+)-alcohol (70). This assignment will be used throughout the rest of this chapter.

4.5.3 OTHER YEAST MEDIATED REDUCTIONS OF KETONE (72)

Fermenting brewer's yeast (active dried yeast, Edme Ltd, England) produced (S)-(+)-(70). There were significant amounts of reduced alcohol (70) present after two hours, by t.l.c. However, the reduction was still incomplete after 4 days.

(72), 1.13g/l

(S)-(+)-(70)
36% yield.
$[\alpha]_D^{25} = +25.2°$

Conditions:
Brewer's yeast,
sucrose (10g),
water (100ml),
room temperature, 4 days.

Scheme 4.15

Seven yeasts were then screened from the laboratory culture collection. They were inoculated into Vogel's medium and cultured as usual (as described in Chapter 3, Section 3.5). Yeast reductions of ketone (72) were attempted at a substrate concentration of 3 mg/ml. Although reduction was observed, by t.l.c., the reactions were extremely slow. The experiment was repeated at a substrate concentration of 1 mg/ml. However, none of the seven yeasts reduced the ketone completely. Another yeast screen was set up of nine different yeasts; the substrate concentration was again 1 mg/ml. No reaction went to completion. However, four yeasts showed almost complete conversion. These four reaction mixtures were worked-up in the usual manner (see Section 3.5). The only difference was that the alcohol (70) was purified by flash chromatography (and not P.L.C.). The results are shown in Table 4.1.

Table 4.1 Results from third yeast screen for the reduction of ketone (72) at a substrate concentration of 1 mg/ml.

Yeast	Time /days	Recovered sm (72) /%yield	Product (70) /%yield	$[\alpha]_D^{25}$	%ee*
Candida guilliermondii ATCC 9058	4	8	67	+14.4	n.d.
Saccharomyces sp. "Sacch"	4	6	61	+13.8	n.d.
Torulopsis keryr 1040	4	9	68	+27.7	98
Torulopsis ernobii ATCC 20,000	4	17	55	-27.7	97

* The %ee was determined by synthesising the corresponding acetate (85) of the alcohol product (70). The acetate (85) was purified by flash chromatography. The %ee of acetate (85) was determined by ^1H n.m.r. (200 MHz) in the presence of *Tris*-3-(heptafluoropropylhydroxymethylene)-d-camphorato], europium (III) derivative [Eu(hfc)$_3$] (40 mol% eq.).[162]

The chiral shift reagent [Eu(hfc)$_3$] shifts the methyl doublet attributable to the C_1 methyl group of the acetate protected alcohol (85) downfield. At 40 mol % [Eu(hfc)$_3$] the two diastereomeric C_1 methyl doublets (of the (85) : [Eu(hfc)$_3$] transient diastereomeric complexes) are baseline separated (> 0.1 p.p.m.), thereby permitting determination of the enantiomeric excess. Using this method 1% of the minor enantiomer of (85) can be detected (i.e. (85) = 98%ee). (For examples of ^1H n.m.r. shift experiments see Section 4.9).

From Table 4.1 two yeasts gave essentially optically pure alcohol (71) of opposite configuration. However, no reaction had gone to completion. Accordingly an attempt was undertaken to find a

yeast that would not only give optically pure alcohol (70) (especially (R)-(-)-(70)), but would also reduce the ketone (72) completely. Thereby the amount of downstream processing of alcohol (70) would be minimised.

In all, 68 yeasts strains were screened. Only when significant amounts of ketone (72) was reduced was the product alcohol (70) isolated. The specific rotation was then measured and used as an initial guide to estimate the enantiospecificity of the reduction. (n.b. for optically pure (R)-(-)-(70); $[\alpha]_D$ = -28 ^{o} (c 1 , $CHCl_3$.)

The following results were obtained from a screen of ten yeasts. The substrate concentration was 1 mg/ml. No reduction was complete (Table 4.2).

Table 4.2 Yeast reduction of 4-(phenylthio)butan-2-one (72) at a substrate concentration of 1mg/ml.

yeast	Time /days	yield (70)/%	$[\alpha]_D^{25}$
Candida lipolytica ATCC 8661	1.2	44	+ 5.9
Torula rosea 1036	2	53	+ 2.9
Cryptococcus masceranus "Ziffer"	2	59	- 4.6
Rhodotorula rubra 1010	3	20	-17.0
Unknown yeast W-50	3	45	- 3.6
Hansenula wingeii NRRL Y-2340-5	6	62	- 1.1
Torulopsis aeria 1399	6	73	+10.8

Perusal of Table 4.2 shows no successful yeasts. Another yeast screen was conducted at the higher substrate concentration of 2 mg/ml. In all cases the reduction was extremely slow. No reaction went to completion. Of the eight yeasts selected only four were worked-up, after 15 days (Table 4.3).

Table 4.3 Yeast reduction of 4-(phenylthio)butan-2-one (72) at a substrate concentration of 2mg/ml.

yeast	Time /days	yield (70)/%	$[\alpha]_D^{25}$ / °
Rhodotorula rubra ATCC 20,129	15	24	+1.5
Hansenula subpelliculosa NRRL-Y-1683	15	27	+7.0
Torulopsis rotundata NRRL-Y-1402	15	20	+3.7
Rhodotorula sp. "Rhod R5"	15	29	+1.0

Since the rate of reduction was so low when the substrate concentration was 2 mg/ml, the next screen of twenty yeasts reverted to using a concentration (72) of 1 mg/ml (Table 4.4). Again no reaction went to completion (n.b. the yields of alcohol (70) in Table 4.4 are too high. Conversely the specific rotation values are therefore, too low. The reason for this error was attributed to a non-volatile impurity present in the h.p.l.c. grade hexanes used in the flash chromatographic isolation of alcohol (70). All other results presented before and after Table 4.4 used a different, clean, batch of hexanes.)

Table 4.4 Yeast reduction of 4-(phenylthio)butan-2-one (72) at a substrate concentration of 1mg/ml. All reactions were worked-up after 3 days.

yeast	yield (70)/%	$[\alpha]_D^{25}$	%ee
Saccharomyces cerevisiae 45	77	+11.8	n.d.
Candida lipolytica 925A	61	-17.4	88
Soil isolate DDT-26	60	+10.0	n.d.
Hansenula saturus NRRL-Y-1304	78	-15.5	>95
Yarrowia lipolytica ATCC 34088	71	-13.7	>95
Saccharomyces fragilis 1017	107	+16.2	n.d.
Hybrid of 1205, *S.lactis* 1205X61056	108	+17.7	n.d.
Torulopsis collioulosa NRRL Y-172	105	+15.3	n.d.
Edna "beer yeast"	79	+12.0	n.d.
Saccharomyces cerevisiae (Red Star)	50	+ 6.5	n.d.

Since in the case of Table 4.4 the specific rotations are not reliable (see note above Table 4.4) the ee in promising cases were determined as described in Section 4.5.2.[162]

From the screening experiments (68 yeasts) most yeasts reduced ketone (72) to give (S)-(+)-alcohol (70). However, four yeasts produced the opposite (R)-(-)-enantiomer of alcohol (70). These four yeasts are listed in Table 4.5.

Table 4.5 Summary of the yeast screening experiments. A table of yeast that stereospecifically produce (R)-(-)-(70).

Yeast	%ee
Torulopsis ernobii ATCC 20,000	> 95*
Hansenula saturus NRRL-Y-1304	> 95
Yarrowia lipolytica ATCC 34088	> 95
Candida lipolytica 925A	88

* repeated twice

4.6 ATTEMPTED SUBSTRATE SCALE-UP OF THE FOUR (R) SELECTIVE YEASTS.

The four yeasts which reduced ketone (72) at a substrate concentration of 1 mg/ml to give predominantly, or exclusively (R)-(-)-alcohol (70) were scaled-up to a substrate concentration of 5 mg/ml. From previous results (Table 4.3) the reductions are known to be very slow when the substrate concentration was increased. Therefore two sets of experiments were set-up with these four yeasts, in side by side reactions. In one set of experiments the ketone (72) was injected neat into the yeast medium. In the other set of experiments the ketone (72) was

pre-dissolved in ethanol (the total ethanol concentration in the yeast medium was 5% v/v).

The ethanol seems to have little effect on the rate of reduction of ketone (72), by t.l.c. In both cases (with or without ethanol) the reduction appeared to terminate after 41 hours. The reaction mixtures were worked-up after 4 days. Only two yeasts produced significant amounts of alcohol (70). The results of these yeasts are given in Table 4.6.

Table 4.6 Scale-up of substrate (72) concentration to 5 mg/ml and the effect of ethanol on the (R) specific yeasts.

yeast	Ethanol yes/no	yield (70)/%	$[\alpha]_D^{25}$ / °	R/S
Torulopsis ernobii ATCC 20,000	no	24	+1.5	S
Torulopsis ernobii ATCC 20,000	yes	30	+7.0	S
Hansenla saturus NRRL-Y-1304	no	15	+3.7	S
Hansenla saturus NRRL-Y-1304	yes	29	+1.0	S

Addition of ethanol increases the yield of alcohol (70), but not dramatically. The most striking observation is that the yeasts now produce the (S)-alcohol-(70). This can be explained in terms of k_{cat}/K_m, [51] where there is more than alcohol dehydrogenase present in the microorganism (see Section 1.2.2).

Since a higher yield was obtained with *Torulopsis ernobii* ATCC 20,000 all further experiments were carried out using this microorganism. We were still interested in trying to increase the substrate (72) concentration whilst retaining the (R) stereoselectivity. To this end, several experiments were

constructed, but none gave any significant advantage. I will briefly summarise the results.

4.7 EFFECT OF TRACE ELEMENTS ON THE STEREOCHEMISTRY OF THE YEAST REDUCTION OF KETONE (70)

Yamada and Nagasawa[158] have shown that the presence or absence of certain elements, or compounds in the growth medium can radically alter the course of microbiologically mediated reactions. This alteration is presumably attributable to induceable enzymes synthesised by the microorganism. Encouraged by Yamada's work the composition of the Vogel's medium, the medium in which all reactions were performed in, was altered. Vogel's medium is a defined medium (Table 4.7).

Table 4.7 Composition of Vogel's medium

Constituent	Quantity /1000ml
Yeast extract	5.0g
Casamino acids	5.0g
Dextrose	40.0g
Na_3citrate.$5.5H_2O$	3.0g
KH_2PO_4	5.0g
NH_4NO_3	2.0g
$CaCl_2.2H_2O$	0.1g
$MgSO_4.7H_2O$	0.2g
Trace elements	0.1ml

Trace element solution

Constituent	g/100ml
Citric acid.$7H_2O$	5.0
$ZnSO_4.7H_2O$	5.0
$Fe(NH_4)_2(SO_4)_2.6H_2O$	1.0
$CuSO_4.5H_2O$	0.25
H_3BO_4	0.05
$NaMoO_4.2H_2O$	0.05
$MgSO_4.H_2O$	0.05

For routine yeast reductions the medium was made up using the above recipe and diluted to one litre using distilled water. Before sterilising the medium, the pH was adjusted to 5.6 - 5.8.

The first change to the Vogel's medium was to alter the amount of

trace elements added from zero to 10X the normal concentration. At high levels of trace elements racemic alcohol (70) was obtained. However, if trace elements were omitted altogether then essentially optically pure (R)-(-)-alcohol-(70) ($[\alpha]_D$ = -26 °) was produced by the *Torulopsis ernobii* ATCC 20,000-mediated reduction of ketone (72).

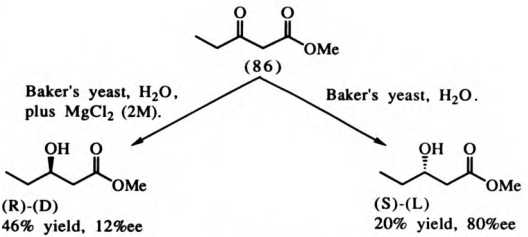
A similar experiment to the one described in Table 4.8 was set up. This experiment was an attempt to increase the amount of (R)-alcohol-(70) produced when the substrate concentration was increased to 5 mg/ml. The reaction was carried out with or without ethanol (Table 4.8). Both reactions were worked-up after 70 hours.

Table 4.8 Scale-up of substrate (72) concentration to 5 mg/ml and the effect of ethanol on the stereospecificity of *Torulopsis ernobii* ATCC 20,000. In this experiment no trace elements solution was present in the Vogel's medium.

yeast	Ethanol yes/no	yield (70)/%	$[\alpha]_D^{25}$ / °	R/S
Torulopsis ernobii ATCC 20,000	no	28	+17	S
Torulopsis ernobii ATCC 20,000	yes	28	+13	S

Unfortunately the desired result was not obtained.

A recent literature report by Ohno *et. al.*[159] showed that the stereochemistry of the baker's yeast reduction of methyl 3-oxopentanoate (86) could be reversed by the addition of magnesium chloride into the yeast medium (Scheme 4.16)



Scheme 4.16

In a similar fashion, although at a much lower concentration, the reduction of ketone (72) was studied by varying the concentrations of Mg^{+2}, Ca^{+2}, SO_4^{-2} and Cl^{-1} ions. Vogel's medium was made-up according to the recipe (Table 4.7) with the exceptions of trace element solutions, $CaCl_2$ and $MgSO_4$. The quantities of calcium chloride and magnesium sulphate was then added according to Table 4.9.

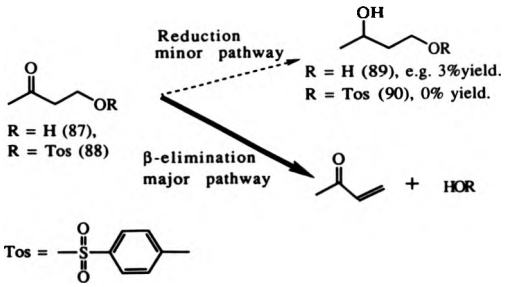
Table 4.9 Effects of modifying the concentration of two "macro" components of the Vogel's medium on the reduction of ketone (72), mediated by *Torulopsis ernobii* ATCC 20,000. Concentration of ketone (72) = 1 mg/ml.

Entry #	$CaCl_2$ g/l	$MgSO_4$ g/l	$MgCl_2$ g/l	sm(72) : p(70) qualitatively, by t.l.c. /2 days
1	X	X	X	100 : 0
2	0.1	0.2	X	5 : 95
3	0.1	X	X	95 : 5
4	X	0.2	X	70 : 30
5	0.2	X	X	70 : 30
6	X	0.4	X	95 : 5
7	X	X	O.2	95 : 5

Entry #2 represents the standard Vogel's medium (except for the absence of trace elements). This is the only medium which gave a sufficiently fast reduction. The product alcohol was isolated (61% yield) $[\alpha]_D$ = -24 °. The results from Table 4.9 are not conclusive. What can be stated positively is that an adequate concentration of both $CaCl_2$ and $MgSO_4$ were required for reduction of ketone (72) to be accomplished by the yeast *Torulopsis ernobii* ATCC 20,000.

4.8 MISCELLANEOUS YEAST REDUCTIONS

Two other related ketones (87) and (88) were tested to see if they could be reduced by a screen of ten yeasts. Both suffered from non-enzymatic β-elimination (Scheme 4.17). No appreciable accumulation of product alcohols (89) and (90) was observed. (Ketone (87) was synthesised by a co-worker {Gu-Ming Guo}. Ketone (87) can be made in one step from butan-1,3-diol (89).[160] The tosylate (88) was also synthesised by Gu-Ming Guo)



Scheme 4.17

However, very recently, Gopalan and Jacobs[161] used baker's yeast to reduce ketone (88) to alcohol (90) in 68% yield, 98%ee, with apparently no problems.

4.9 DETERMINATION OF %EE OF ALCOHOL (70)

The ee of alcohol (70) was determined indirectly as described in Section 4.5.2. Overleaf are some examples of the ^1H n.m.r (200 MHz) spectra obtained. The spectra are of the alcohol (70) when it was protected as its acetate (85) in the presence of [Eu(hfc)$_3$] (40 mol% eq.).[162] Figure 4.1 (a), (b), and (c) show the acetate protected (R)-alcohol product from the yeast *Torulopsis ernobii* ATCC 20,000. Figure 4.2 (a), (b), and (c) show the acetate protected (S)-alcohol product from the yeast *Torulopsis ketyr* 1304.

4.10 SUMMARY

(1) (3E)-4-(Phenylthio)but-3-en-2-one (73) was reduced by yeasts. The olefinic double bond of (73) was reduced initially.

(2) 4-(Phenylthio)butan-2-one (72) was reduced to (2S)-4-(phenylthio)butan-2-ol (70) by *Saccharomyces cerevisiae*.

(3) Most yeasts produced (S)-(+)-(70).

(4) Four yeasts produced (R)-(-)-(70). The best yeast was *Torulopsis ernobii* ATCC 20,000.

(5) The maximum substrate (72) concentration permissible was 1 mg/ml. Modification of the conditions had little effect.

FIGURE 4.1 (a),(b) and (c) Methyl region of the ^1H n.m.r. (200 MHz) of (2R)-4-(Phenylthio)-2-(acetoxy)butane (> 98%ee) in the presence of $Eu(hfc)_3$ (40 mol% equivalent). The acetate (85) is the protected (2R)-alcohol after the yeast (*Torulopsis ernobii* ATCC 20,000) mediated reduction of 4-(Phenylthio)butan-2-one:

(a) 2(R)-4-(Phenylthio)-2-(acetoxy)butane >98%ee,

(b) Expansion of (a), and

(c) (a) plus racemic 2(S,R)-4-(Phenylthio)-2-(acetoxy)butane.

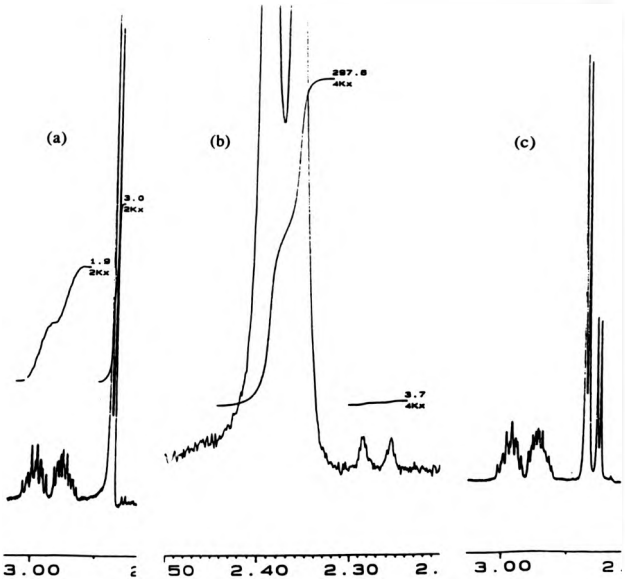
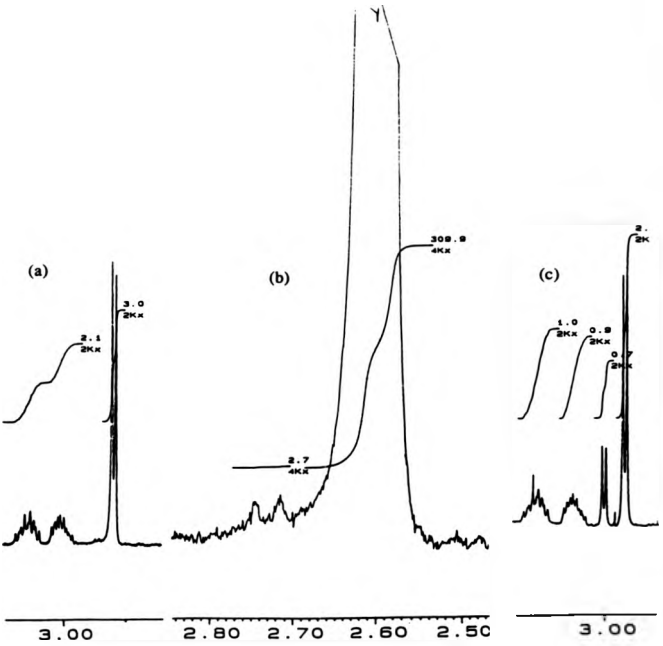


FIGURE 4.2 (a),(b) and (c) Methyl region of the ^{1}H n.m.r. (200 MHz) of (2S)-4-(Phenylthio)-2-(acetoxy)butane (> 98%ee) in the presence of Eu(hfc)$_3$ (40 mol% equivalent). The acetate (85) is the protected (2S)-alcohol after the yeast (*Torulopsis ketyr* 1040) mediated reduction of 4-(Phenylthio)butan-2-one:

(a) 2(S)-4-(Phenylthio)-2-(acetoxy)butane >98%ee,

(b) Expansion of (a), and

(c) (a) plus racemic 2(S,R)-4-(Phenylthio)-2-(acetoxy)butane.



CHAPTER FIVE

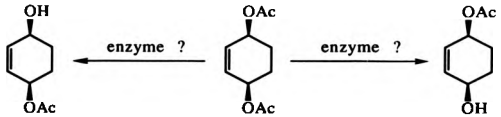
The preparation of *cis*-3-hydroxy-6-acetoxycyclohexene (79%ee).

5.1 INTRODUCTION

From a practical point of view *meso* compounds are ideal substrates for hydrolytic enzymes. The reactions can produce optically active compounds. In theory, given an ideal enzyme, the product can be obtained enantiomerically pure, in quantitative yield (see Section 1.3.1).[17,18,61,62,63]

5.2 AIMS

This project had the straightforward aim of producing the *cis*-hydroxyacetate (*cis*-3-hydroxy-6-acetoxycyclohexene) (93) in an optically pure state. We aimed to achieve this goal by enantiospecifically hydrolysing the *cis*-diacetate (*cis*-3,6-diacetoxycyclohexene) (94) (Scheme 5.1).

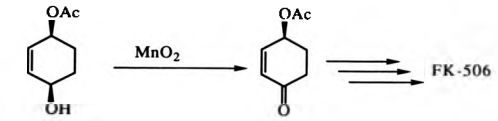


(3S,6R)-(93) (94) (3R,6S)-(93)

Scheme 5.1

The hydroxyacetate (93) could be a useful building block for organic synthesis. For example, allylic oxidation of (3R,6S)-(93) would produce (4S)-acetoxycyclohex-2-en-1-one (95) (Scheme

5.2). Compound (95) could then be used in Danishefsky *et. al.* synthesis of the immunosuppressant FK-506 (Scheme 5.2).[164] Danishefsky's group obtained an analogue of (S)-(95) in a six step synthesis starting from (D)-(-)-quinic acid.[165]

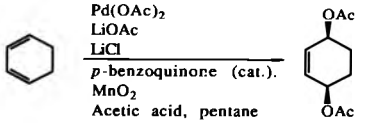


(3R,6S)-(93) (95)

Scheme 5.2

5.3 SYNTHESIS OF *MESO* DIACETATE (94)

Cis-3,6-Diacetoxycyclohexene was readily prepared in a one pot reaction following the method of Bäckvall *et. al.* (Scheme 5.3).[166]



(94), >96% *cis*

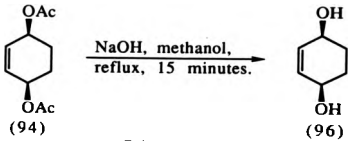
Scheme 5.3

The reaction is both regio- and stereoselective to give *cis*-diacetate (94) (>96% *cis*). Pure *cis*-diacetate (94) was isolated by flash chromatography. If LiCl was omitted from the reaction

mixture, Bäckvall *et. al.* established that *trans*-diacetate (94) was formed preferentially.[166]

5.4 SYNTHESIS OF CHEMICAL STANDARDS

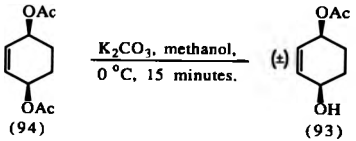
Cis-2-Cyclohexene-1,4-diol (96) was synthesised by basic hydrolysis of diacetate (94) as described by Bäckvall *et. al.* (Scheme 5.4).[166]



(94) (96)

Scheme 5.4

A sample of racemic *cis*-(3SR, 6RS)-3-hydroxy-6-acetoxycyclohexene (93) was synthesised by basic hydrolysis (Scheme 5.5). The optimal conditions for the maximum yield of hydroxyacetate (93) are given below.



(94) (93)

Scheme 5.5

Hydroxyacetate (93) was isolated by flash chromatography in 47% yield. Interestingly, when the diol (96) was treated under acetylation conditions (1.1 mol equiv. acetic anhydride, pyridine, CH_2Cl_2, DMAP (cat.)) only diol (96) and diacetate (94) were observed by t.l.c.

5.5 ELECTRIC EEL CHOLINESTERASE, ACETYL CATALYSED HYDROLYSIS OF *MESO* DIACETATE (94)

The hydrolysis of diacetate (94) catalysed by electric eel cholinesterase, acetyl (EC 3.1.1.7) (EEA) resulted in essentially racemic hydroxyacetate (93) (Scheme 5.6).

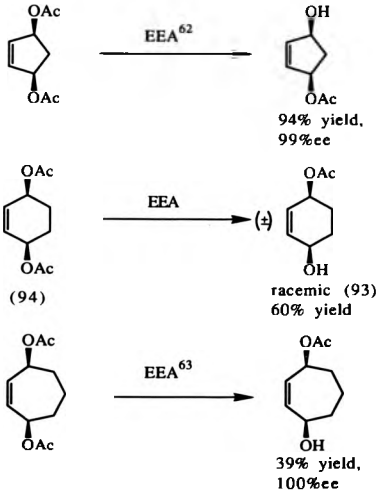
(93), 60% yield,
$[a]_D^{22}$ = -0.63 (c 1.2, $CHCl_3$)

Conditions:
EEA = 0.5 mg, 50 units,
200mM phosphate buffer, pH 7, 2ml.
Room temperature, 2 hours.

Scheme 5.6

For optically pure hydroxyacetate (93) the specific rotation will be between 85 ° and 95 °. This value has been obtained by correlating the $[\alpha]_D$ values with %ee determinations from various optically active samples of hydroxyacetate (93) (see Sections 5.6 and 5.10).

The results using electric eel cholinesterase, acetyl clearly follows a distinct trend for this enzyme (Scheme 5.7).[62,63]

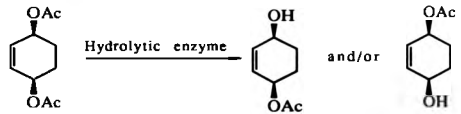


Scheme 5.7

5.6 A SCREEN OF OTHER HYDROLYTIC ENZYMES

Twenty three enzymes were screened for their ability to hydrolyse the diacetate (94). Only enzymes which produced hydroxyacetate (93) as the major product were worked-up. The hydroxyacetate was isolated by flash chromatography. The results are summarised in Table 5.1.

Table 5.1 Enzyme screening results for the attempted stereospecific formation of hydroxyacetate (93).



(94), 50 mg (+)-(3S,6R)-(93) (-)-(3R,6S)-(93)

#	Enzyme source and code	Time /hrs	yield /%	$[\alpha]_D$ /°	ee* /%
1	*Aspergillus niger* lipase AP	2	27	+16.0	
2	*Candida cylindracea* lipase AY	2	37	+29.7	
3	*Chromobacterium viscosum* lipase CV	2	51	+42.8	47
4	Pig liver esterase (PLE)	7	59	-43.5	49
5	*Pseudomonas sp.* lipase K-10	7	47	+37.3	
6	*Alicaligenes sp.* lipase PL	27	43	-23.6	
7	Porcine pancreatic lipase (PPL)	27	37	-19.2	
8	Electric eel cholinesterase, acetyl	2	60	- 0.6	
9	*Candida rugosa* lipase	4	27	+47.1	
10	*Pseudomonas sp.* lipase AK	4	19	+36.5	
11	*Candida cylindracea* lipase MY	4	60	+35.1	
12	*Pseudomonas sp.* lipase P-30	4	64	+70.0	79
13	Subtilisin type XXIV	6	38	-34.6	

Enzyme suppliers are given in the Section 7.5.

*ee was determined by ^1H n.m.r. ($CDCl_3$, 300 MHz) in the presences of the chiral solvating agent (R)-(-)-2,2,2-trifluoro-1-(9-anthryl)ethanol (2 mol eq.).[174] Under these conditions the acetate singlet of the hydroxyacetate (93) was split into two singlets which could be successfully integrated (see Section 5.10).

From Table 5.1 the best enzyme, in terms of both yield and enantiomeric excess of product (93), was lipase P-30 *ex*

Pseudomonas sp. (Amano Pharmaceutical Company). Lipase P-30 produced (+)-(3S,6R)-(93) in 64% yield, 79%ee (this represents an enantiomer ratio of 9 : 1). In all subsequent biotransformations described in this chapter this enzyme was used.

First, we wished to obtain the (-)-enantiomer of (93), using lipase P-30.

5.7 LIPASE P-30 IN THE ESTERIFICATION DIRECTION

As described in Section 1.3.2 enzymes can be used in low water systems. Usually the stereochemical "sense" of the enzyme is preserved.[109] Therefore access to the other enantiomer is available by changing the solvent. The *cis* diol (96) was acetylated under irreversible conditions, using isopropenyl acetate and lipase P-30 (Scheme 5.8).[78]

(96), 30mg

(-)-(3R,6S)-(93)
62% yield,
$[\alpha]_D = -32^\circ$ (c 2.55 $CHCl_3$)
(estimated to be *ca.* 35%ee)

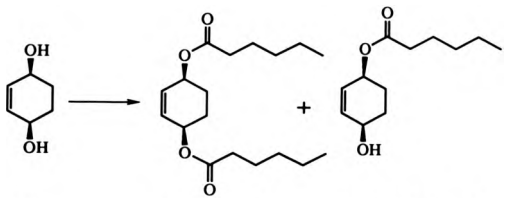
Conditions:
Dry isopropenyl acetate = 4 mol eq.
Dry THF = 2ml,
Lipase P-30 = 50mg,
46 hours, room temperature.

Scheme 5.8

An attempt to improve the optical purity of hydroxyacetate (93) by modifying the substrate was tried.[71]

5.8 SUBSTRATE MODIFICATION OF *CIS*-DIACETATE (94)

The *cis*-dihexanoate (97) of the *cis*-diol (96) was synthesised, using Steglich's esterification methodology.[108] Some racemic *cis*-monohexanoate (98) was also isolated (Scheme 5.9).

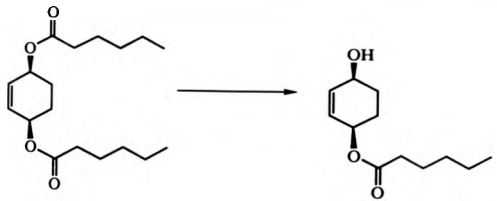


(96) (97), 41%yield (±) (98), 25% yield

Conditions:
Hexanoic acid = 2 mol eq., dry CH_2Cl_2, dicyclohexylcarbodiimide, DMAP (cat.), 0 °C -> room temperature.

Scheme 5.9

Hydrolysis of the *cis*-dihexanoate catalysed by lipase P-30 was extremely slow (Scheme 5.10).

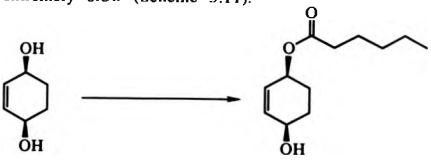


(97) (98)

Conditions:
Lipase P-30, phosphate buffer 200mM pH 7, R.T.

Scheme 5.10

Similarly attempted esterification of *cis*-diol (96) was also extremely slow (Scheme 5.11).[22]



(96) (98)

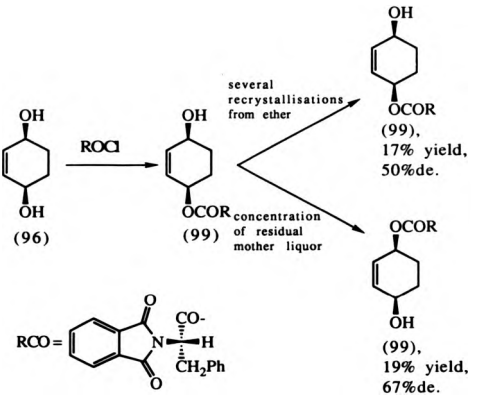
Conditions:
Lipase P-30, hexanoic acid (5 mol eq.),
isooctane, 65 °C.

Scheme 5.11

So in this case substrate modification did not lead to improved results (compare with Section 2.8.2). However, it must be remembered that Schemes 5.10 and 5.11 represent only initial experiments.

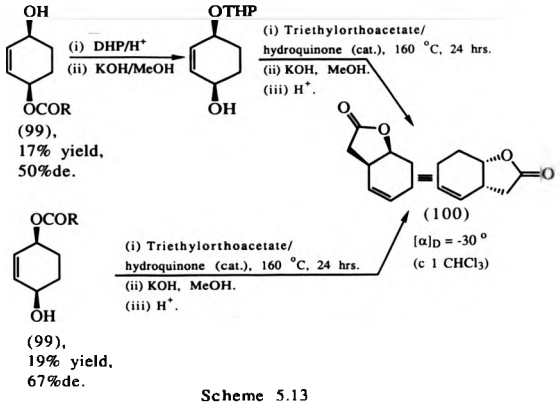
5.9 DETERMINATION OF THE ABSOLUTE STEREOCHEMISTRY OF HYDROXYACETATE (93)

Terashima, Nara and Yamada formed diastereomeric diesters (99) of *cis*-diol (96).[167] They partially resolved the diastereomers. One of the diastereomers selectively crystallised from ether (Scheme 5.12).



Scheme 5.12

Both diastereomers of (99) were converted into the optically active bicyclic lactone (100). The reactions were enantio-convergent, leading to only one enantiomer of lactone (100) (Scheme 5.13). Repeated recrystallisation gave optically pure lactone (100) in 15% overall yield.[167]

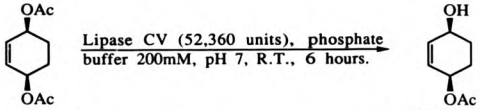


Scheme 5.13

The lactone (100) had previously been synthesised by Corey and Snider ($[\alpha]_D$ = -28 ° (c 0.83, $CHCl_3$)).[168] Lactone (100) is a central intermediate in the synthesis of prostaglandin analogues.[167,168,169,170]

Accordingly, to determine the stereochemistry of the enzymatic hydrolysis of *cis*-diacetate (94). The product hydroxyacetate (93) was converted into the lactone (100), in an analogous manner to Scheme 5.13.

Firstly the hydrolysis of *cis*-diacetate (94) catalysed by lipase CV (*ex Chromobacterium viscosum*) was scaled-up (Scheme 5.14).

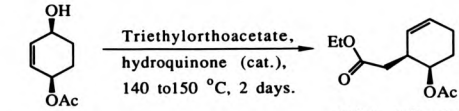


(94), 1.02g

(+)-(3S,6R)-(93)
90% yield, $[a]_D = +36^{\circ}$
ee *ca* 42% (from rotation)

Scheme 5.14

The product hydroxyacetate (+)-(93) was then converted *via* a Claisen rearrangement into the intermediate diester (101) (Scheme 5.15).[167,170,171,172,173]

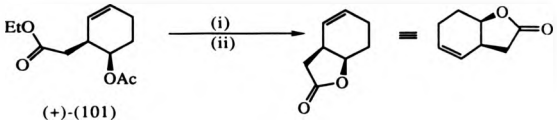


(+)-(3S,6R)-(93)

(101), 44% yield,
$[a]_D = +24.5^{\circ}$
(c 1.28, $CHCl_3$)

Scheme 5.15

The diester (101) was isolated by flash chromatography in 44% yield. Diester (101) was then hydrolysed ($KOH/MeOH/H_20$). The resultant potassium salt was neutralised and without isolation lactonised, under acid catalysis, to yield the lactone (100) (Scheme 5.16).



(+)-(101)

(+)-(100), 70% yield,
$[\alpha]_D = +9.2^{\circ}$ (c 2.12, MeOH)

Conditions:
(i) KOH (3.6) mol eq., MeOH : H_2O ; 9 : 1, 18 hours.
(ii) *Para*-toluenesulphonic acid (cat.), EtOAc : THF ; 3 : 1.

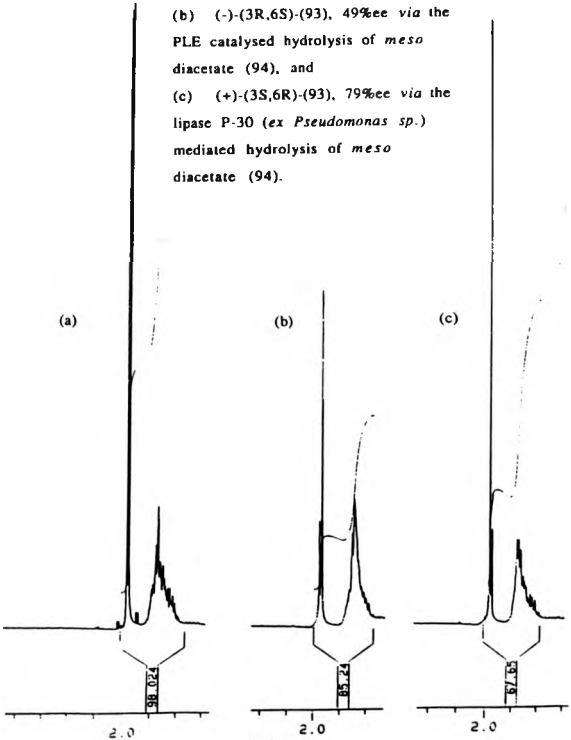
Scheme 5.16

The (+)-lactone-(100)[169] was isolated by flash chromatography in 70% yield. Thus the stereochemistry of the hydroxyacetates (93) in Table 5.1 was assigned.

5.10 DETERMINATION OF THE %EE OF HYDROXYACETATES (93)

All ^1H n.m.r. methods based on chiral shift reagents failed. The Mosher's ester derivative of hydroxyacetate (93) was synthesised.[103] However, no splitting was observed by ^1H n.m.r. (up to 300 MHz). Splitting was observed by ^{19}F n.m.r. (282 MHz), but the singlets were not baseline resolved. The method of choice was to add two mol eq. of the chiral solvating agent[174] (R)-(-)-2,2,2-trifluoro-1-(9-anthryl)ethanol. In a $CDCl_3$ solution, at ^1H n.m.r. (300 MHz), the acetate singlets of the two transient diastereomeric complexes are well resolved. Some examples are given in Figure 5.1 (a), (b), and (c).

FIGURE 5.1 (a),(b) and (c) Acetate region of the ^{1}H n.m.r. (300 MHz) spectra of *Cis*-3-Hydroxy-6-acetoxycyclohexene (93) in the presence of (R)-(-)-2,2,2-Trifluoro-1-(9-anthryl)ethanol (2 mol equivalents): (a) (3SR,6RS)-(93), (b) (-)-(3R,6S)-(93), 49%ee *via* the PLE catalysed hydrolysis of *meso* diacetate (94), and (c) (+)-(3S,6R)-(93), 79%ee *via* the lipase P-30 (*ex Pseudomonas sp.*) mediated hydrolysis of *meso* diacetate (94).



5.11 SUMMARY

(1) Twenty three enzymes were screened for their ability to hydrolyse stereospecifically *cis*-3,6-diacetoxycyclohexene.

(2) Lipase P-30 from *Pseudomonas sp.* gave the best result. It produced (+)-(3S,6R)-3-hydroxy-6-acetoxycyclohexene (93) in 64% yield, 79%ee.

(3) The (-)-(3R,6S)-(93) enantiomer was obtained by using the same enzyme in the reverse, esterification direction.

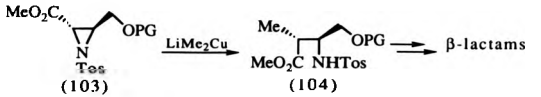
(4) Substrate modification had little effect.

CHAPTER SIX

Chemoenzymatic approaches to chiral aziridine-2-carboxylates.

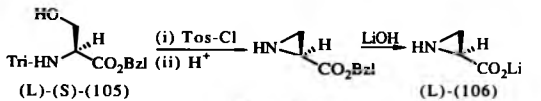
6.1 INTRODUCTION

Aziridines, like their oxygen analogues epoxides, are versatile intermediates for use in chemical synthesis. Chiral aziridines have many uses.[176] Functionalised aziridines (e.g. 103) have been regioselectively opened by organocuprates (Gilman reagents) to form intermediates (e.g. 104) useful in the synthesis of β-lactams (Scheme 6.1).[177]



Scheme 6.1

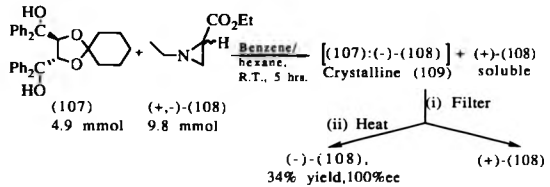
Optical pure aziridine-2-carboxylic acid, lithium salt (106) has been synthesised from a protected form of serine (105) (Scheme 6.2).[178]



Scheme 6.2

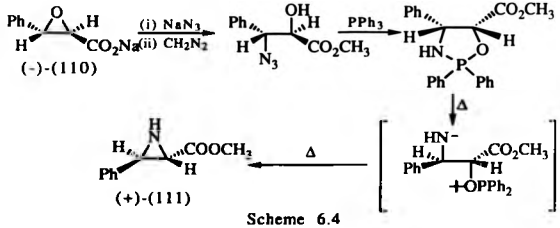
Direct resolution of racemic chiral aziridines are rare.[176] However, a recent report[176] described the elegant resolution of various substituted aziridines. Mori and Toda enantioselectively formed a

one to one host-guest complex (109) which crystallised from solution, leaving the opposite enantiomer of the chiral aziridine ((+)-(108)) behind in solution. Heating the complex (109) liberated (-)-aziridine-(108) in an optically pure state (Scheme 6.3).



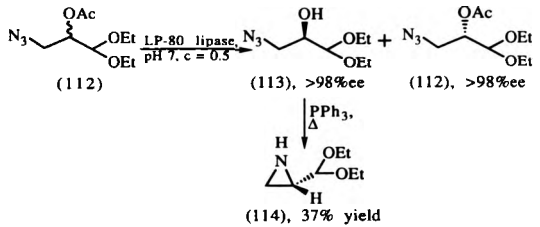
Scheme 6.3

Several indirect resolution methods to chiral aziridines have recently appeared in the literature.[179,180,181,182,183] Zwanenburg *et. al.*[179] resolved all four diastereomers of epoxide (110) *via* classical chemical resolution in eighteen steps. The epoxide (110) was then converted into optically pure aziridine-2-carboxylate ester (111) (Scheme 6.4).



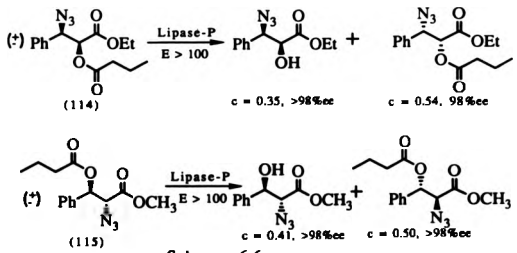
Scheme 6.4

Wong *et. al.*[180] resolved, enzymatically, 2-acetoxy-3-azidopropanal diethylacetal (112). The product azido alcohol (113) was converted, *via* a similar sequence as shown in Scheme 6.4, into chiral aziridine (114) (Scheme 6.5).



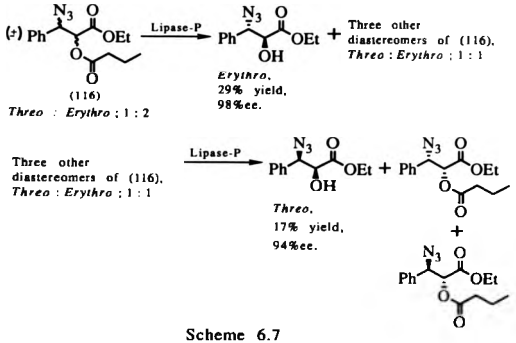
Scheme 6.5

Three papers published by Honig *et. al.* [181,182,183] resolved α-azido alcohols, as their corresponding butyrates. In the first paper simple azido butyrates were resolved.[181] Honig then resolved azido butyrates (114) and (115) with an additional ester functionality present in the molecule, using lipase P (*ex Pseudomonas sp.*) (Scheme 6.6).



Scheme 6.6

He extended this idea and directly resolved enantiomers from a *threo* and *erthryo* mixture of azido butyrate (116) (Scheme 6.7),[183] again using lipase P.

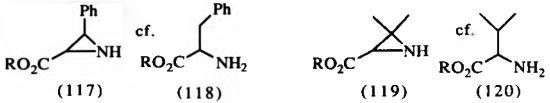


Scheme 6.7

All the azido alcohols produced in Schemes 6.6 and 6.7 have the potential to be converted into chiral aziridines *via* the triphenylphosphine route (see Schemes 6.4 and 6.5).[179,180,184]

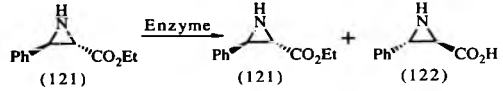
6.2 AIMS

The aim of this project was to produce optically pure aziridine carboxylic ester analogues of amino acids. For example, in Scheme 6.8 aziridine ester (117) is the analogue of phenylalanine (118). Also in Scheme 6.8 aziridine ester (119) is the formal equivalent of valine (120).



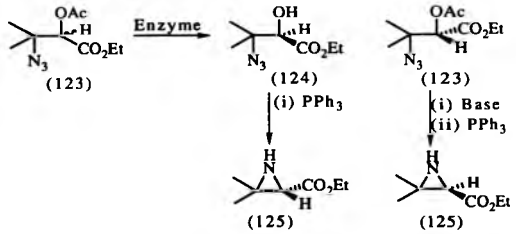
Scheme 6.8

We aimed to produce these aziridines either by direct resolution of racemic aziridine-2-carboxylic acid esters (121) (Scheme 6.9),



Scheme 6.9

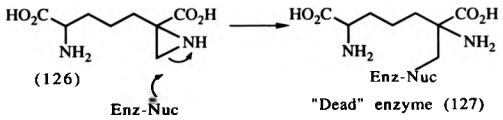
or *via* resolution of the α-azido acetate ester (123), followed by chemical conversion to the aziridine-2-carboxylic acid ester (125) (Scheme 6.10).[179,180,184]



Scheme 6.10

We envisaged that these chiral aziridines could potentially be new chemotheraputic agents.

Higgins *et. al.* recently demonstrated that recombinant diaminopimelate epimerase in *E. coli* can be irreversibly inhibited by the aziridine (126) (Scheme 6.11).[185]



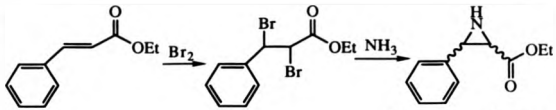
Scheme 6.11

Similarly, Umazawa *et. al.* showed that (2SR,3SR)-2,3-dicarboxyaziridine was a competitive inhibitor of aspartase (*ex E. coli*).[186]

Aziridine-2-carboxylic esters have been successfully incorporated into synthetic peptides by the group of Okawa *et. al.* [178,187,188, 189,190,191] Ede *et. al.* incorporated an unusual amino acid into a peptide and observed unusual properties when compared to the natural peptide.[192] We therefore imagined that incorporation of aziridine-2-carboxylic ester into a given peptide sequence may mask the reactive aziridine ring, and act like a "suicide" substrate at a specific receptor (compare with Scheme 6.11).

6.3 SYNTHESIS AND ENZYME HYDROLYSIS OF ETHYL 3-PHENYL-1H-AZIRIDINE-2-CARBOXYLATE

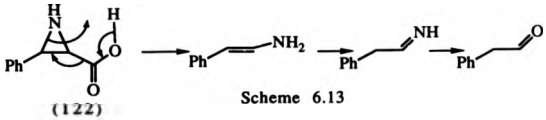
A chromatographically separable *cis*/*trans* (*cis*:*trans*;1:1) mixture of ethyl 3-phenyl-1H-aziridine-2-carboxylate (121) was synthesised following literature procedures (Scheme 6.12).[193,194]



(128) (129) (121) *cis* and *trans* separated by F.C.

Scheme 6.12

A previous worker (Dr A. Dachs) in our group attempted the resolution of racemic *trans*-(121) and racemic *cis*-(121). (For structures of *cis*-and *trans*-(121)[194] see Table 6.1). She observed that only *trans*-(121) was a substrate for the protease α-chymotrypsin. *Trans*-(121) was isolated, after incubation with α-chymotrypsin, in 42% yield and 85%ee (%ee determined at ^1H n.m.r. (400 MHz) in the presence of 3 mol eq. of the chiral solvating agent (S)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol[174]). The hydrolysed product *trans*-3-phenyl-1H-aziridine-2-carboxylic acid (122) was not isolable. Aziridine-2-carboxylic acids are known to be labile to acids,[178] presumably they lose CO_2 by β-elimination, followed by rearrangement and hydrolysis (Scheme 6.13).

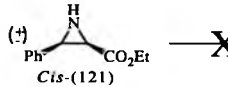


(122)

Scheme 6.13

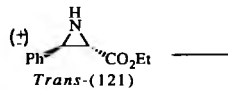
A screen of nine enzymes was set-up in an attempt to find an enzyme that would hydrolyse the *cis*-aziridine ester (121). The same enzymes were also tested against *trans*-(121) (Table 6.1).

Table 6.1 Attempted resolution of racemic *cis*-(121) and racemic *trans*-(121) using hydrolytic enzymes.



Cis-(121)

Conditions:
cis-(121) (50mg) pre-dissolved in CH_3CN (0.2ml), enzyme (10-20mg), phosphate buffer 100mM, pH 7, 2ml.



Trans-(121)

Conditions:
trans-(121) (50mg), enzyme (10-20mg), phosphate buffer 100mM, pH 7, 2ml.

Enzyme	*Cis*-(121) hydrolysis	*Trans*-(121) hydrolysis	Rate of *Trans*-(121) hydrolysis	Is the reaction yellow ?
Subtilisin carlsberg (type VII)	X	yes	++	yes
Papain (type III)	X	X		X
Trypsin (type II)	X	yes	++	yes
Protease S	X	X		X
Protease A2	X	yes	+++	yes
Lipase *ex Chromobacterium viscosum*	X	yes	+	yes
alpha-chymotrypsin (type II)	X	yes	++++	yes
Lipase *ex Pseudomonas fluorescens*	X	yes	++++	yes
Lipase *ex Candida cylindracea*	X	yes	+++	yes
Blank	X	X		X

The reactions were monitored directly by t.l.c. An attempt was made to make a sample of the free acid (122). Racemic *cis*-(121) was reacted with one equivalent of LiOH[178] and a yellow solid was isolated. However, attempts to isolate the free acid (121) failed. Accordingly the reactions were monitored crudely by the disappearance of the ester u.v. active spot and appearance of u.v. activity on the baseline. In the experiments were hydrolysis was

observed the reaction mixture turned yellow. The rate of hydrolysis of *trans*-(121) is given qualitatively by:

Rate of hydrolysis of *trans*-(121)	Ratio of ester (121) to acid (122) after 20 hours, very qualitative.
+	95 : 5
++	80 : 20
+++	60 : 40
++++	<(50 : 50)

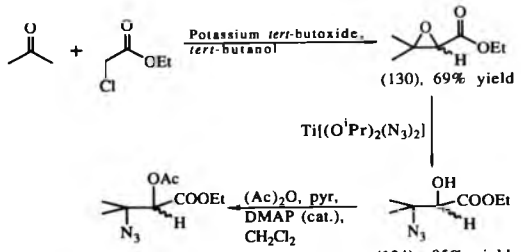
The isolation of the free aziridine-carboxylic acid (122) caused problems. Only the *trans*-ester (121) could be hydrolysed. As this work was in progress, a route to all the enantiomers of the methyl esters of acid (122) was published.[179] It was decided to terminate this approach to aziridines (121) and (122).

6.4 SYNTHESIS OF VALINE AZIRIDINE ANALOGUE

This approach was more successful. We decided to approach the aziridine (119) by the synthetic pathway outlined in Scheme 6.10.

The synthesis of azido acetate (123) is given in Scheme 6.14. Firstly ethyl 3-methyl-2,3-epoxy-butanoate (ethyl 3,3-dimethylglycidate)[195] (130) was synthesised by a Darzens condensation of acetone and ethyl chloroacetate. The resulting epoxide (130) was regioselectively opened using $Ti[(O^iPr)_2(N_3)_2]$[196,197] to produce the azido alcohol (124). This regioselective ring opening was first achieved by Dr A. Dachs. The azido alcohol was acetylated directly to produce the azido acetate

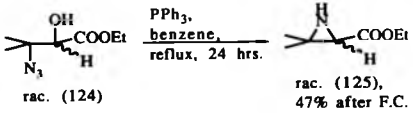
$Ti[(O^iPr)_2(N_3)_2]$[196,197] to produce the azido alcohol (124). This regioselective ring opening was first achieved by Dr A. Dachs. The azido alcohol was acetylated directly to produce the azido acetate (123), which was purified by flash chromatography (Scheme 6.14).



Scheme 6.14

6.5 SYNTHESIS OF RACEMIC ETHYL 3,3-DIMETHYL-1H-AZIRIDINE-2-CARBOXYLATE (125)

Following the procedure of Zwanenberg *et. al.*[179] racemic azido alcohol (124) was converted to aziridine (125) (Scheme 6.15).

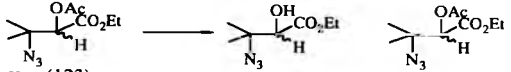


Scheme 6.15

6.6 ENZYME CATALYSED RESOLUTION OF AZIDO ACETATE (123)

Nine commercial lipases were screened for their ability to hydrolyse racemic azido acetate (123). The enantioselectivity of the resolution was established by determining the E value for a given resolution.[22] The results from the eight enzymes worked-up are given in Table 6.2.

Table 6.2 Lipase screen for the stereoselective hydrolysis of azido acetate (123)



rac. (123), *ca.* 40mg (-)-(124) (+)-(123)

Condtions:
Lipase (*ca.* 20 mg), phosphate buffer, pH 7, 100mM (2ml), R.T.

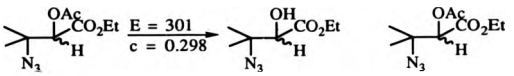
Lipase *ex*	Time /hrs	c	%ee (124)	%ee (123)	E
Pseudomonas fluorescens	7	0.34	92	48	39
Chromobacterium viscosum	30	0.19	76	19	9
Porcine pancreatic lipase	4	0.04	95	4	41
Mucor mehei	48	0.23	67	20	6
Candida cylindracea	2.5	0.47	>98	88	584
Rhizopus languinosa	24	0.34	81	42	30
Penicillium roqueforti	336	0.19	87	20	17
Aspergillus niger	2.5	0.52	77	83	20

The ee of the product azido alcohol (124) was established by firstly converting the alcohol to its corresponding (R)-Mosher's ester (131),[103] followed by ^{1}H n.m.r. (400 MHz) analysis (see Section 6.7).

ester (131),[103] followed by ^1H n.m.r. (400 MHz) analysis (see Section 6.7).

The ee of the azido acetate (123) was measured directly by ^1H n.m.r. (400 MHz) in the presence of the chiral solvating agent ((S)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol, 1 mol eq.[174]) (see Section 6.8).

The best enzyme was the lipase from *Candida cylindracea* (*ex* Biocatalysts Ltd). That reaction was scaled-up (Scheme 6.16).



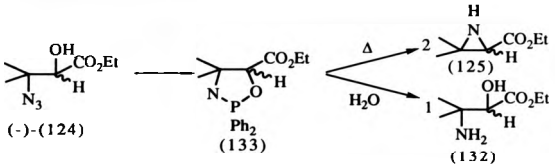
rac (123),
2.04g,
8.9 mmol

(-)-(124)
31% yield,
>98%ee,
$[\alpha]_D^{24}$ = -13.5 °
(c 1.03, $CHCl_3$)

(+)-(123)
69% yield,
42%ee,
$[\alpha]_D^{24}$ = +13.6 °
(c 0.98, $CHCl_3$)

Scheme 6.16

The product azido alcohol (-)-(124) was converted into aziridine (125) as in Scheme 6.15. The aziridine (125) was isolated by flash chromatography (Scheme 6.17). However, ^1H n.m.r. analysis showed another compound was present. The impurity could possibly be the amino-alcohol (132). The amino-alcohol could have arisen from incomplete conversion of the oxaphosholidine (133) to aziridine, and subsequent hydrolysis of intermediate (133) (Scheme 6.17).



Scheme 6.17

$[a]_D^{24}$ = -30.4 °
(c 0.75, $CHCl_3$)

The problem with this reaction may be circumvented by simply distilling the reaction mixture.[180] This would probably completely convert oxaphosholidine (133) into aziridine (125). The route to aziridine (125) and determination of the absolute configuration requires further investigation.

6.7 DETERMINATION OF THE %EE OF AZIDO ALCOHOL (124)

The %ee was determined by converting the azido alcohol (124) into its corresponding (R)-Mosher's ester (131).[103] ^1H n.m.r. (400 MHz) analysis revealed two well resolved sets of signals. One set was attributable to the methoxy protons of the (R)-Mosher's ester and resonated at δ = 3.65 p.p.m. and 3.54 p.p.m respectively. The other set of singlets was attributable to the methine proton at C-2 (of the original azido alcohol (124)). The two diastereomeric protons resonated at δ = 4.89 p.p.m and 4.85 p.p.m. respectively. Thus an internal check on the %ee was established. Some examples of the of the ^1H n.m.r. (400 MHz) spectra are given overleaf in Figure 6.1 (a) and (b) and Figure 6.2 (a) and (b).

FIGURE 6.1 (a) and (b) ^1H n.m.r. (400 MHz) spectra of the Mosher's ester (131) of racemic Ethyl,3-methyl-3-azido-2-hydroxybutanoate: (a) Methine region, and (b) Methoxy region.

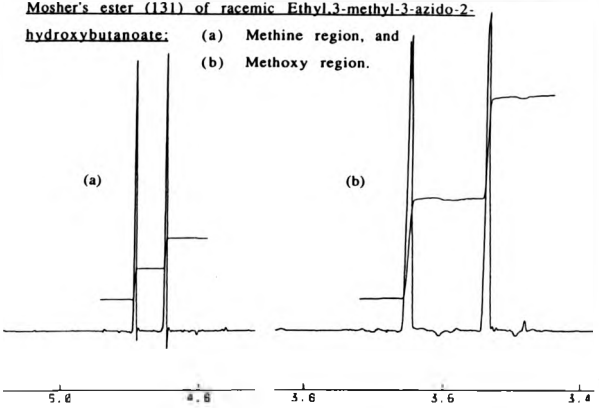
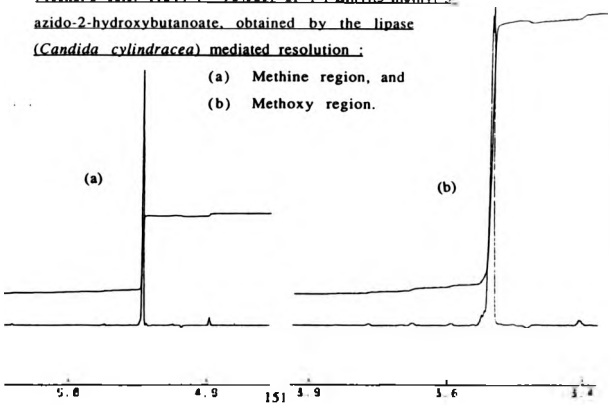


FIGURE 6.2 (a) and (b) ^1H n.m.r. (400 MHz) spectra of the Mosher's ester (131) (> 98%de) of (-)-Ethyl,3-methyl-3-azido-2-hydroxybutanoate, obtained by the lipase (*Candida cylindracea*) mediated resolution : (a) Methine region, and (b) Methoxy region.



6.8 DETERMINATION OF THE %EE OF THE RESIDUAL AZIDO ACETATE (123)

The ee of the azido acetate (123) was established directly at ^1H n.m.r. (400 MHz) in the presence of one mol eq. of the chiral solvating agent ((S)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol in a C_6D_6 solution.[174] All of the proton resonances of azido acetate (123) were split, except for the acetate singlet. The largest splitting (> 0.02 p.p.m.) were observed for the methine proton at C-2 and the two methyl group protons at C-3 and C-4. Five sets of singlets were baseline resolved. Some examples are given in Figures 6.3 (a) and (b), Figures 6.4 (a) and (b), and Figures 6.5 (a) and (b) overleaf.

6.9 SUMMARY

(1) Direct resolution of aziridine-2-carboxylic ester (121) was not totally successfully. Only *trans*-(121) was hydrolysed.

(2) Resolution of azido acetate (123) was successful.

(3) The lipase from *Candida cylindracea* resolved azido acetate (123) with high degree of stereoselectivity (E >100).

(4) A route from azido alcohol (124) to aziridine (125) was established. However, some work still needs to be carried out to make the reaction reproducible. The absolute configuration has not been established.

FIGURE 6.3 (a) and (b) ^{1}H n.m.r. (400 MHz) spectra of racemic Ethyl,3-methyl-3-azido-2-(acetoxy)butanoate in the presence of (S)-(+)-2,2,2-Trifluoro-1-(9-anthryl)ethanol (1 mol equivalent):

(a) Methine region, and

(b) Methylene region.

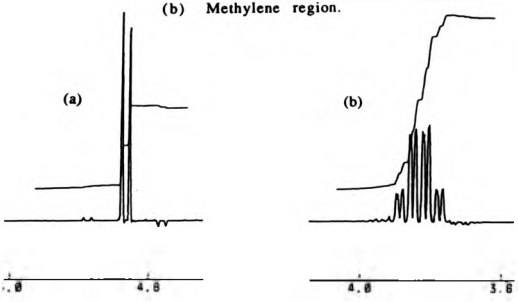


FIGURE 6.4 (a) and (b) ^{1}H n.m.r. (400 MHz) spectra of (+)-Ethyl,3-methyl-3-azido-2-(acetoxy)butanoate (88%ee) in the presence of (S)-(+)-2,2,2-Trifluoro-1-(9-anthryl)ethanol (1 mol equivalent):

(a) Methine region, and

(b) Methylene region.

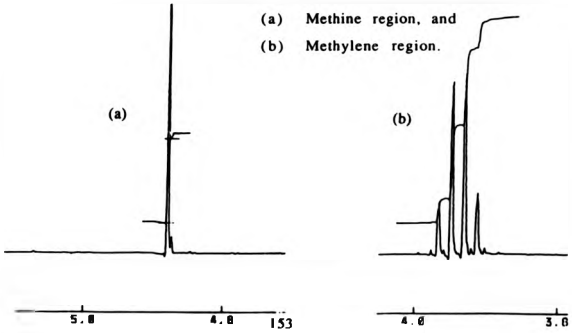
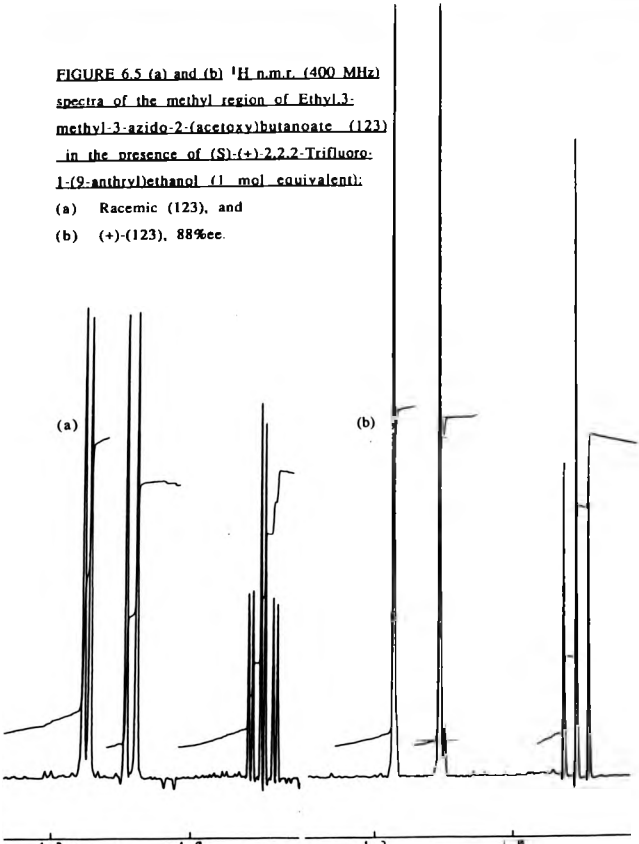


FIGURE 6.5 (a) and (b) ^1H n.m.r. (400 MHz) spectra of the methyl region of Ethyl,3-methyl-3-azido-2-(acetoxy)butanoate (123) in the presence of (S)-(+)-2,2,2-Trifluoro-1-(9-anthryl)ethanol (1 mol equivalent):

(a) Racemic (123), and

(b) (+)-(123), 88%ee.



CHAPTER SEVEN-EXPERIMENTAL DETAILS

7.1 INTRODUCTION

The experimental details are reported according to the instructions for authors of The Journal of The Chemical Society.[198] Chemicals were either purified following literature methods,[199] or purchased as the highest available grade.

Nuclear magnetic resonance spectra were recorded using the following instruments:

Bruker WH-400 (operating frequency ^1H n.m.r. = 400 MHz),
Perkin-Elmer R-34 (operating frequency ^1H n.m.r. = 220 MHz),
Bruker WM-200 (operating frequency ^1H n.m.r. = 200 MHz),
Bruker 300 (operating frequency ^1H n.m.r. = 300 MHz), and
Jeol GX-270 (operating frequency ^1H n.m.r. = 270 MHz).

All are fourier transform spectrometers (except for the Perkin-Elmer R-34, which is a continuous wave spectrometer). ^{13}C n.m.r. were recorded at an operating frequency of 100 MHz. Chemical shifts are reported in δ relative to trimethylsilane = 0.00 ppm). Multiplicities of ^1H n.m.r. signals are abbreviated as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. All other multiplets are written in full (e.g. quintet).

T.l.c. were run on glass plates pre-coated with 0.20mm or 0.25mm Merck silica gel 60_{F254}. Detection used one or more of the following methods:[200,201]
U.V. fluorescence quenching.

Phosphomolybdic acid (7g/l), followed by heating.

Cerium sulphate (2.5g) in water (160ml) and H_2SO_4 (48ml) followed by heating.

Ninhydrin (0.2%) in *n*-butanol : acetic acid; 95 : 5, and heating.

2,4-Dinitrophenylhydrazine (0.4g) in 2M HCl (100ml) and heating.

50% H_2SO_4 and charring at 200 °C.

Bromocresol green (0.04g) in ethanol (100ml), to which was added 0.1M NaOH until the blue colour disappears.

Para-Anisaldehyde (1ml) in H_2SO_4 (1ml) and ethanol (18ml), heat.

Exposure to iodine vapour.

PLC (preparative layer chromatography) were run on 20cm x 20cm glass plates, pre-coated with either 1mm or 2mm Merck silica gel 60_{F254}.

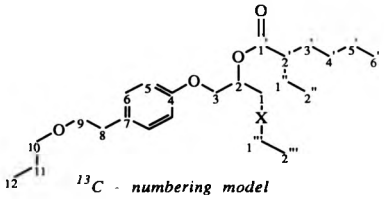
FC (Flash Chromatography), all columns contained silica to a height of 15-20 cm. FC was performed according to the method of Still *et al*[202] The following solvents were glass distilled for use in FC and PLC: Light petroleum ether (b.p. = 40-60 °C), ethyl acetate, diethyl ether.

Optical rotations were measured on either an Optical Activity Limited, Model AA-1000 polarimeter (in a 2 dm pathlength cell), or a Perkin-Elmer, Model 241-C polarimeter (pathlength = 1 dm). Concentration c = g/ 100ml, wavelength = 589 nm. Melting points are uncorrected. Gas-liquid chromatography (g.l.c.) using packed columns (1.8 m in length) used either a PYE 104 or a PYE 204 instrument. Gas-liquid chromatography (g.l.c.) using capillary columns were run on a Carlo-Erba Fractovap 2450 series instrument. Nitrogen was the carrier gas at a flow rate of 30 ml/min. Infra red spectra were recorded on either a Perkin-Elmer 580-B or a

Perkin-Elmer 1720X machine. Mass spectrum were recorded on either a Kratos MS 80 or a Finnigan 4000 instrument.

7.2 EXPERIMENTAL DETAILS FOR CHAPTER TWO.

Assignments of ^{13}C spectra use the numbering system in the following model of the carbon skeleton:



^{13}C - numbering model

3-[4-[2-Hydroxyethyl]phenoxy]propene (16) - To a stirred solution of 2-[4-hydroxyphenyl]ethanol (15) (10.0g, 72mmol) in AR grade acetone (30ml), was added anhydrous potassium carbonate (15.9g, 57mmol) and allyl bromide (7.5ml, 86mmol). The suspension was boiled under reflux for 18 hours, (g.l.c., SE30[10%], 158 °C, R_t (15) = 4min, R_t (16) = 6min), the reaction mixture was diluted with water (40ml), extracted with dichloromethane (2x40ml), washed with aqueous 5%w/v NaOH (2x40ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure. Kugelrohr distillation (oven temperature 110 °C/0.1mmHg) yielded (16) as a colourless liquid (9.66g, 75%yield), homogeneous by g.l.c and ^1H n.m.r.; δ_H (220 MHz; solvent $CDCl_3$; standard Me_4Si) 1.52 (1H, bs, O-*H*), 2.81 (2H, t, *J* 7Hz, ArCH_2), 3.83 (2H, bt, CH_2OH), 4.53 (2H, d, *J* 6Hz, CH_2OAr), 5.28 (1H, d, *J* 10.3Hz, *CH*H=CH (*cis*)), 5.42 (1H, d, *J* 19.1Hz, *CH*H=CH (*trans*)), 6.08 (1H, m, *CH*=CH_2),

6.88 (2H, d, J_{ortho} 8.3 Hz, Ar-*H*), and 7.15 (2H, d, J_{ortho} 8.3Hz, Ar-*H*); δ_C (100MHz; solvent $CDCl_3$) 37.9 (C-8), 63.3 (C-3), 68.5 (C-9), 114.5 (C-5), 117.1 (C-1), 129.6 (C-6), 130.6 (C-2), 133.1 (C-7), and 156.9 (C-4). m/z (EI) 178 (M^+, 31%), 147 ($(M-CH_2OH)^+$, 100), and 107 (56); HRMS, found (m/z): 178.0989, $C_{11}O_2H_{14}$ requires: 178.0993.

(±)-3-[4-[2-Hydroxyethyl]phenoxy]-1,2-epoxypropane (17).- To a stirred solution of 2-[4-hydroxyphenyl]ethanol (15) (40g, 0.289mol) in dry butan-2-one (100ml) was added anhydrous potassium carbonate (37.6g, 0.376mol) and freshly distilled epibromohydrin (27.3ml, 0.319mol). The suspension was boiled under reflux for 24 hours, (g.l.c., SE30[10%], 200 °C, R_t (15) = 4min, R_t (17) = 9min). The suspension was cooled, filtered and evaporated under reduced pressure to yield (17) as a yellow oil which solidified on standing. Recrystallisation (ethyl acetate/light petroleum (b.p. 40-60 °C)) yielded (17) as a white solid (51.6g, 92%yield), homogeneous by g.l.c. and ^1H n.m.r.; ν_{max} (nujol mull) 3 215 (b, OH), 1 620 (m, Ar), 1 515 (s, Ar), 1 300 (m), 1 250 (s, epoxide), 910 (m, epoxide), and 830 cm^{-1} (m); δ_H (220 MHz; solvent $CDCl_3$; standard Me_4Si) 1.53 (1H, bs, O-*H*), 2.72-2.83 (4H, m, ArCH_2 and terminal epoxide protons), 3.37 (1H, m, methine epoxide proton), 3.83 (2H, t, *J* 7.5 Hz, CH_2OH), 3.96 (1H, dd, J_{gem} -11 Hz and J_{anti} 6 Hz, ArC*H*H (*anti*)), 4.22 (1H, dd, J_{gem} -11Hz and J_{syn} 3 Hz, ArOC*H*H (syn)), 6.89 (2H, d, J_{ortho} 9 Hz, Ar-*H*), and 7.17 (2H, d, J_{ortho} 9Hz, Ar-*H*); m/z (EI) 194 (M^+, 70%), 176 ($(M-H_2O)^+$, 6), 163 (96), 133 (19), and 107 (100); m/z (Cl NH_3) 212 ($(M+NH_4)^+$, 16%); HRMS, found (m/z): 194.0944, $C_{11}O_3H_{14}$ requires: 194.0943.

3-[4-[2-Acetoxyethyl]phenoxy]propene (18).- To a stirred solution of 3-[4-[2-hydroxyethyl]phenoxy]propene (16) (1.0g, 5.6mmol) in dry pyridine (15ml), at 0 °C under nitrogen, was added slowly dry acetyl chloride (0.4ml, 5.6mmol) and the mixture was allowed to come to room temperature during

18 hours, (g.l.c., SE30(3%), 150 °C, R_t (16) = 6.5min, R_t (18) = 11.5min). The suspension was filtered and evaporated under reduced pressure. The residue was dissolved in dichloromethane (20ml), washed with an aqueous saturated solution of sodium hydrogen carbonate (20ml), dilute sulphuric acid (20ml), and water (10ml), dried ($MgSO_4$), filtered and evaporated at reduced pressure. Kugelrohr distillation (oven temperature 115 °C/0.3mmHg) yielded (18) as a colourless liquid (0.57g, 46% yield), homogeneous by g.l.c. and ^1H n.m.r. pure; δ_H (220 MHz; solvent $CDCl_3$; standard Me_4Si) 2.08 (3H, s, C(O)CH_3), 2.92 (2H, t, J 7.3 Hz, ArCH_2), 4.30 (2H, t, J 7.3 Hz, ArCH_2CH_2), 4.58 (2H, d, J 5 Hz, CH_2OAr), 5.35 (1H, d, J 10 Hz, CHH=CH (*cis*)), 5.48 (1H, d, J 18 Hz CHH=CH (*trans*)), 6.13 (1H, m, CH_2=CH), 6.93 (2H, d, J_{ortho} 9 Hz, Ar-H), and 7.20 (2H, d, J_{ortho} 9 Hz, Ar-H); δ_C (100MHz; solvent $CDCl_3$) 20.5 (C-11), 34.0 (C-8), 64.8 (C-3), 78.5 (C-9), 114.4 (C-5), 117.1 (C-1), 129.3 (C-6), 129.6 (C-2), 133.2 (C-7), 157.0 (C-4), and 170.4 (C-10); m/z (EI) 220 (M^+, 2.6%), 161 (18), 147 (15), 160 ((M-acetate)$^+$, 100), 119 (46), and 107 (17); HRMS, found (m/z): 220.1099; $C_{13}O_3H_{16}$ requires: 220.1098.

(±)-3-[4-[2-Acetoxyethyl]phenoxyl]1,2-epoxypropane (19).

Method A.- To a stirred solution of 3-[4-[2-acetoxyethyl]phenoxy]propene (18) (0.20g, 0.91mmol) in dry dichloromethane (2ml) was added *meta*-chloroperbenzoic acid (0.21g, 1.2mmol). The reaction mixture was stirred for nine days. (g.l.c., SE30(3%), 172 °C, R_t (18) = 4min, R_t (19) = 11min, also an extra peak present). The solution was diluted with dichloromethane (10ml), washed with 10%w/v NaOH (10ml), back extracted with dichloromethane (2x10ml), washed with water (10ml), dried ($MgSO_4$), filtered and evaporated at reduced pressure. Kugelrohr distillation (oven temperature 140 °C/1mmHg) gave (19) as a colourless liquid (0.034g, 17% yield), ^1H n.m.r. identical to ^1H n.m.r. data reported for (19) synthesised by **Method B**.

Method B.- To a stirred ice cold solution of 3-[4-[2-hydroxyethyl]phenoxy]1,2-epoxypropane (17) (17.08g, 83mmol) in dry THF (100ml) was added dry triethylamine (11.53ml, 83mmol) followed by dry acetyl chloride (5.83ml, 82mmol). The reaction mixture was stirred at 0 °C for 30 mins and room temperature for 40 hours. (G.l.c., SE30{3%}, 205 °C, R_t (17) = 5min, R_t (19) = 8.5min). The reaction mixture was diluted with dichloromethane (50ml), washed with 1M HCl (2x100ml), saturated sodium hydrogen carbonate solution (100ml), and water (100ml), to yield an orange oil (19) (18.57g, 90% yield). An analytical sample was purified by PLC (toluene : diethyl ether; 1 : 1). The band at R_f = 0.7 was recovered; δ_H (220 MHz; solvent $CDCl_3$; standard Me_4Si) 2.05 (3H, s, C(O)CH_3), 2.85-2.95 (4H, m, ArCH_2 and terminal epoxide protons), 3.37 (1H, m, epoxide methine proton), 3.98 (1H, dd, J_{gem} -11Hz and J_{anti} 5.4 Hz, ArOC*H*H (*anti*)), 4.20-4.32 (3H, m, ArOC*H*H (*syn*) and CH_2OAc), 6.92 (2H, d, J_{ortho} 8.8 Hz, Ar-*H*), and 7.18 (2H, d, J_{ortho} 8.8 Hz, Ar-*H*); m/z (EI) 176 ((M-acetate)$^+$, 100%); m/z (EI) 236 (M+, 2%), 176 (87%); m/z (CI NH_3) 254 ((M+NH_4)$^+$, 13%), 177 ((M+1-acetate)$^+$, 16); HRMS, found (m/z): 236.1039, $C_{13}O_4H_{16}$ requires: 236.1049.

Attempted enzymatic epoxidation of alkenes by *Methylococcus capsulatus* (Bath)

A crude enzyme extract from *Methylococcus capsulatus* (Bath) was prepared by Dr. J. Green (Department of Biological Sciences, University of Warwick) following the method of Dalton *et. al.*[95] The enzyme extract was stored as pellets in phosphate buffer (20mM, pH 7) at -20 °C.

Methylococcus capsulatus (Bath)-catalysed epoxidation of allyl chloride.- Crude enzyme extract (1ml) was placed in a 10ml conical flask and sealed with a suba-seal. The extract was defrosted and then heated to 45 °C on a

shaker water-bath for one minute. Then simultaneously, *via* two syringes, allyl chloride (10µl, 0.12mmol) and 0.1M NADH, in phosphate buffer, pH 7, 20mM (50µl, 0.5µmol) was added. The reaction was shaken at 45 °C. After 3 minutes an aliquot (10µl) was directly analysed by g.l.c., (g.l.c., SE30{3%}, 25 °C, R_t (allyl chloride) = 4mins, R_t (allyl alcohol) = 6mins, and R_t (epichlorohydrin) = 22mins). All three compounds were identified by injecting standards and co-injection. The yield (by g.l.c.) of epichlorohydrin was 5±2%. The ee of the epichlorohydrin was determined by chiral g.l.c. The reaction mixture was extracted with diethyl ether (1ml). The organic phase was placed in a small sample vial. Then a very gently flow of nitrogen gas was used to reduce the volume to 0.25ml. 5µl was then injected onto the chiral g.l.c. column. (50m x 22mm, internal volume = 1.6ml, coated with 0.125M Nickel *bis* 3-(heptafluorobutyrl)-(1R)-camphorate on an OV 101 support; 74 °C, R_t (one enantiomer) = 24mins, R_t (other enantiomer) = 28mins. Enantiomeric excess epichlorohydrin = 0±5%.

Methylococcus capsulatus (Bath)-catalysed epoxidation of allyl bromide.- The same procedure as above was used. The quantities used are as follows:- allyl bromide (14µl, 0.16mmol), 0.1M NADH (50µl). (In another experiment allyl bromide (14µl) and 0.1M NADH (200µl) was used, however, little effect on the amount of epibromohydrin produced was observed.). After 3 minutes an aliquot (10µl) was injected onto the g.l.c. column (g.l.c., PEG 20M, temperature program: initial isotherm = 66 °C for 10mins, then a temperature rise of 5 °C/ min. Final isotherm = 140 °C, R_t (allyl bromide) = 5mins, R_t (allyl alcohol) = 17mins, and R_t (epibromohydrin) = 25mins). The components were identified by injection of standards, co-injection and also GC-MS. The yield of epibromohydrin (by g.l.c.) was 5±2%. GC-MS (carbowax g.l.c. column same temperature program as above R_t (allyl bromide) =

5min; m/z (EI) 122 (M^+, 78%), 120 (M^+, 78), 41 ($allyl^+$, 100). R_t (H_2O) = 13-14min; m/z (EI) 18 (M^+, 100). R_t (allyl alcohol) = 15min; m/z (EI) 58 (M^+, 70), 57 ($(M-1)^+$, 100). R_t (epichlorohydrin) = 25min; m/z (CI NH_3) 156 ($(M+NH_4)^+$, 8%), 154 ($(M+NH_4)^+$, 8), 58 (31), 52 (37), 35 (100).

Attempted *Methylococcus capsulatus* (Bath)-catalysed epoxidation of 3-[4-[2-hydroxyethyl]phenoxy]propene (16).- The same procedure as above was followed. The quantities used were (16) (18µl, 0.1mmol), 0.1M NADH (50µl, in another experiment 4x50µl). Reaction followed by g.l.c., (g.l.c., SE30{10%}, 155 °C, R_t (16) = 6.5mins, R_t (18) = 17mins). No reaction was observed.

(±)-3-[4-[2-acetoxyethyl]phenoxy]-1-chloropropan-2-ol (20).- Into a solution of (±)-3-[4-[2-acetoxyethyl]phenoxy]-1,2-epoxypropane (19) (2.7g, 11mmol) in dry dichloromethane (20ml) was bubbled dry HCl gas for 90 minutes. (T.l.c. (toluene : diethyl ether; 1 : 1), R_f (19) = 0.54, R_f (20) = 0.48). The solvent was evaporated under reduced pressure to yield (20) as a colourless oil (2.31g, 78% yield), homogeneous by t.l.c. and ^1H n.m.r. An analytical sample was purified by PLC (toluene : diethyl ether; 1 : 1); δ_H (220 MHz; solvent $CDCl_3$; standard Me_4Si) 2.05 (3H, s, $C(O)CH_3$), 2.90 (2H, t, J 7.3 Hz, Ar-CH_2), 3.14 (1H, bs, OH), 3.37 (2H, m, CH_2OAr), 4.09 (2H, d, J 5.4 Hz, CH_2Cl), 4.17-4.31 (3H, m, $ArCH_2CH_2$ and CHOH), 6.89 (2H, d, J_{ortho} 9 Hz, Ar-H), 7.18 (2H, d, J_{ortho} 9 Hz, Ar-H); δ_C (100MHz; solvent $CDCl_3$) 20.4 (C-11), 33.6 (C-8), 45.6 (C-1), 64.8 (C-3), 68.3 (C-2), 69.5 (C-9), 114.2 (C-5), 129.5 (C-6), 130.6 (C-7), 156.7 (C-4), and 170.4 (C-10); m/z (EI) 274 (M^+, 2.4%), 272 (M^+, 6.7), 215 ($(M-acetate)^+$, 24), 214 (79), 213 ($(M-acetate)^+$, 63), and 212 (99); m/z (CI NH_3) 292 ($(M+NH_4)^+$, 4.3%), 290 ($(M+NH_4)^+$, 12.2), 215 ($(M-acetate)^+$, 24), 214 (68), 213 ($(M-acetate)^+$, 70), 212 (100); HRMS, found (m/z): 274.0778,

$C_{13}O_4H_{17}{}^{37}Cl$ requires: 274.0789; Found (m/z): 272.0799, $C_{13}O_4H_{17}{}^{35}Cl$ requires: 272.0815.

3-[4-[2-Acetoxyethyl]phenoxy]-1-chloropropan-2-one (21) - To an ice cold stirred solution of (±)-3-[4-[2-acetoxyethyl]phenoxy]-1-chloropropan-2-ol (20) (2.00g, 7.4mmol) was added 2.67 mol Cr^{+3} dm^{-3} Jones reagent (11.1mmol Cr^{+3}, 4.16ml) and left to come to room temperature. The reaction mixture was stirred for 18 hours. (T.l.c., (toluene : diethyl ether; 1 : 1) R_f (20) = 0.48, R_f (21) = 0.62). The reaction was about 95% complete. The reaction mixture was diluted with water (100ml), and the solution was extracted with diethyl ether (3X100ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure. FC (150g silica, toluene : diethyl ether; 9 : 1) gave a white solid which was recrystallised ($CHCl_3$/light petroleum ether (b.p. 40-60 °C)) to yield (21) as white crystals (1.49g, 75% yield) m.p.= 45.5-47 °C, homogeneous by t.l.c. and ^1H n.m.r.; δ_H (220 MHz; solvent $CDCl_3$; standard Me_4Si) 2.06 (3H, s, C(O)C*H*$_3$), 2.92 (2H, t, *J* 7 Hz, Ar-*H*), 4.28 (2H, t, *J* 7.3 Hz, ArCH$_2$C*H*$_2$), 4.46 (2H, s, ArOC*H*$_2$), 4.80 (2H, s, C*H*$_2$Cl), 6.87 (2H, d, J_{ortho} 8.8 Hz, Ar-*H*), and 7.22 (2H, d, J_{ortho} 8.8 Hz, Ar-*H*); m/z (Cl NH_3) 290 ((M+NH_4)$^+$, 27%), 288 ((M+NH_4)$^+$, 83), 212 ((M+NH_4-acetate)$^+$, 13), and 210 ((M+NH_4-acetate)$^+$, 32). HRMS, (Cl NH_3) (m/z) found: 290.0983, $[C_{13}O_4{}^{37}ClH_{15}.NH_4]^+$ requires: 290.0973; (Cl NH_3) (m/z) found: 288.1003, $[C_{13}O_4{}^{35}ClH_{15}.NH_4]^+$ requires: 288.1002.

3-[4-[2-Acetoxyethyl]phenoxy]-1-chloro-2(R,S)-[(S)-2'-methoxy-2'-trifluoromethylphenylacetoxy]propane (23).[103] - A solution of (±)-3-[4-[2-acetoxyethyl]phenoxy]-1-chloropropan-2-ol (20) (0.112g, 0.41mmol), dry pyridine (2ml), dichloromethane (2ml) and (R)-(-)-2-methoxy-2-trifluoromethylphenylacetylchloride (0.156g,

0.60mmol) was stirred at room temperature for 20mins. (T.l.c., (light petroleum ether (b.p. 40-60 °C) : ethyl acetate; 1 : 1) R_f (20) = 0.39, R_f (23) = 0.56. The suspension was diluted with water (20ml), the solution was extracted with chloroform (3x20ml), washed with 1M HCl (20ml), saturated sodium carbonate solution (20ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure. Purification by PLC (light petroleum ether (b.p. 40-60 °C) : ethyl acetate; 1 : 1), the band at R_f 0.7 yielded (23) as a colourless liquid (0.124g, 62% yield); δ_H (220 MHz; solvent $CDCl_3$; standard Me_4Si) 2.02 (3H, s, C(O)CH_3), 2.88 (2H, t+t, *J* 7.3 Hz, ArCH_2), 3.56 (1.5H, s, OCH_3), 3.60, (1.5H, s, OCH_3), 3.74-3.92 (2H, m, CH_2OAr), 4.10-4.32 (4H, m, CH_2Cl and ArCH_2CH_2), 5.60 (1H, m, CHCH$_2$Cl), 6.75-6.90 (2H, m, Ar-*H*), 7.10-7.28 (2H, m, Ar-*H*), 7.40 (3H, m, Ar*H* 5' and 6'), and 7.56 (2H, m, Ar-*H* 4').

Baker's yeast mediated reduction of 3-[4-[2-acetoxyethyl]phenoxy]-1-chloropropan-2-one (21).- A suspension of baker's yeast (Sainsbury's) (10g), warm distilled water (100ml) and sucrose (10g) was stirred at room temperature for 10 minutes. To the fermenting suspension was added (21) (0.10g, 0.58mmol dissolved in ethanol (1ml)). After 17 hours all the starting material had been consumed. Several other spots were visible by t.l.c., (t.l.c., (toluene : diethyl ether; 1 : 1) R_f (21) = 0.59, R_f (20) = 0.49). Celite® (10g) was added to the reaction mixture, and the mixture was stirred then filtered through a pad of Celite®. The aqueous filtrate was extracted with diethyl ether (3x50ml), washed with saturated sodium hydrogen carbonate solution (50ml), and water (50ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was purified by PLC (diethyl ether : toluene; 1 : 1) to yield a colourless liquid (20) (0.013g, 13% yield). The ^{1}H n.m.r. of the yeast reduction product (20) is the same as authentic racemic (20). The ee was determined by synthesising the corresponding Mosher's ester (23) (as described above, making sure that the rection had gone to

completion by t.l.c. before work-up) followed by ^1H n.m.r (400 MHz) analysis. The ee (20) = 25±2%.

(±)-3-[4-[2-Hydroxyethyl]phenoxy]-1-chloropropan-2-ol (24).- Into a solution of (±)-3-[4-[2-hydroxyethyl]phenoxy]1,2-epoxypropane (17) (1.01g, 5.2mmol) in dry dichloromethane (20ml) was bubbled dry HCl gas for 10 minutes. (T.l.c., (toluene : diethyl ether; 1 : 2) R_f (17) = 0.4, R_f (20) = 0.3). The reaction mixture was evaporated at reduced pressure. FC (100g silica, toluene : diethyl ether; 1 : 2) yielded (24), as a colourless liquid (1.02g, 86% yield), homogeneous by ^1H n.m.r. and t.l.c.; δ_H (270 MHz; solvent $CDCl_3$; standard Me_4Si) 2.84 (2H, t, J 7 Hz, Ar-CH_2), 3.06 (2H, bs, O*H* x2), 3.66-3.86 (4H, m, CH_2OAr and CH_2OH), 4.06 (2H, m, CH_2Cl), 4.18 (1H, m, *CH*OH), 6.86 (2H, d, J_{ortho} 9 Hz, Ar-*H*), and 7.16 (2H, d, J_{ortho} 9 Hz, Ar-*H*). m/z (EI) 232 (M^+, 33%), 230 (M^+, 75), 201 ($(M-CH_2OH)^+$, 44), 199 ($(M-CH_2OH)^+$, 100), 163 ($(M-HCl)^+$, 28).

3-[4-[2-((S)-1]'-methoxy-1]'-trifluoromethylphenylacetoxy)ethyl] phenoxy]-1-chloro-2(R,S)-[(S)-2']-methoxy-2'-trifluoromethyl phenylacetoxy]propane (25).- A solution of (±)-3-[4-[2-hydroxyethyl]phenoxy]-1-chloropropan-2-ol (24) (0.020g, 0.087mmol), (R)-(-)-2-methoxy-2-trifluoromethylphenylacetylchloride (0.055g, 0.219mmol), carbon tetrachloride (2ml) and dry pyridine (2ml) was stirred at room temperature for 20 minutes, under nitrogen. The reaction was terminated before completion. (T.l.c., (toluene : diethyl ether; 1 : 1) R_f (24) = 0.2, R_f (mono Mosher's ester) = 0.3, and R_f (*bis*-Mosher's ester (25)) = 0.9). The suspension was diluted with dichloromethane (20ml), washed with 1M HCl (20ml), saturated sodium carbonate solution (20ml), dried ($MgSO_4$) filtered and evaporated under reduced pressure. The residue was purified by PLC (toluene : diethyl ether; 1 : 1). The band at Rf (0.5) was

identified by ^1H n.m.r to be a mono Mosher's ester (12mg, 30% yield), the acylation had occured at the primary hydroxyl of diol (24); δ_H (270 MHz; solvent $CDCl_3$; standard Me_4Si) 2.52 (1H, d, *J* 5.7 Hz, *OH*), 2.84 (2H, t, *J* 7 Hz, Ar-CH_2), 3.46 (3H, s, OCH_3), 3.76 (2H, m, $ArOCH_2$), 4.06 (2H, m, CH_2Cl), 4.22 (1H, m, *CH*OH), 4.50 (2H, t, J 7 Hz, $ArCH_2CH_2$), 6.82 (2H, d, J_{ortho} 9 Hz, Ar-*H*), 7.11 (2H, d, J_{ortho} 9 Hz, Ar-*H*), and 7.20-7.46 (5H, m, Ar-*H*).

The band at Rf (0.9) was recovered and filtered through a short column of silica to yield (25) as a colourless liquid (28mg, 49% yield); δ_H (270 MHz; solvent $CDCl_3$; standard Me_4Si) 2.96 (2H, m, Ar-CH_2), 3.48 (3H, s, OCH_3 C-11(H)), 3.58 (1.5H, s, OCH_3 C-2'(H)), 3.62 (1.5H, s, OCH_3 C-2'(H)), 3.72-3.98 (2H, m, $ArOCH_2$), 4.08-4.26 (2H, m, CH_2Cl), 4.49 (2H, bt, $ArCH_2CH_2$), 5.61 (1H, m, *CH*O(MPTA)), 6.80 (2H, dd, J_{ortho} 8.5 Hz, Ar-*H*), 7.18 (2H, apparent triplet, *J* 8.5 Hz, Ar-*H*), 7.22-7.46 (8H, m, Ar-*H*), and 7.80 (2H, m, Ar-*H*); m/z (EI) 662 (M^+, =3%), 430 ((M-Mosher's acid)$^+$, 10), 428 ((M-Mosher's acid)$^+$, 33), 309 (100), 189 (95), 105 (28), 77 (19).

Microbiological reduction of 3-[4-[2-acetoxyethyl]phenoxy]-1-chloropropan-2-one (21).- These reduction experiments were performed by E. Tidswell (Department of Microbiology and Botany, University College of Wales, Aberystwyth). To a growing culture of microorganism was added 3-[4-[2-acetoxyethyl]phenoxy]-1-chloropropan-2-one (21) (0.040g, 0.175mmol). The reduction was monitored by t.l.c., (toluene : diethyl ether; 1 : 1), R_f (21) = 0.62, R_f (20) = 0.48, R_f (24) = 0.40. After work-up the major product appeared to be 3-[4-[2-hydroxyethyl]phenoxy]-1-chloropropan-2-ol (24), by t.l.c. The desacetoxy diol (24) was isolated by PLC (toluene : diethyl ether; 1 : 1) and subsequently converted into the *bis*-Mosher's ester (25) (as described above, making sure that the reaction was complete by t.l.c. before work-up). (25) was isolated after work-up by PLC (light petroleum ether (b.p. 40-60 °C) : diethyl ether; 1 : 1) followed by filtering

through a short column of silica (1g silica, (light petroleum ether (b.p. 40-60 °C) : diethyl ether; 2 : 1). The full results are given in the following table:

microorganism	microorganism Carbon source	yield / %	ee / %	diastereomer A/B ?
Lactobacillus brevis	?	17	29	A
Clostridium pasteurianum	?	19	60	B
Clostridium kluyveri	ethanol + acetate	18	88	B
Clostridium tyrobutyrium LA1	glucose	trace	n.d.	n.d.
Clostridium tyrobutyrium LA1	crotonate	13	60	B

(±)-3-[4-[2-Acetoxyethyl]phenoxy]-1-chloro-2-butanoyloxypropane (26).- To a stirred solution of (±)-3-[4-[2-acetoxyethyl]phenoxy]-1-chloropropan-2-ol (20) (2.00g, 7.4mmol), dry pyridine (5ml), dry dichloromethane (20ml) and 4-dimethylaminopyridine (cat.), was added distilled butanoic anhydride (1.5ml, 9.2mmol). (T.l.c., (toluene : diethyl ether; 1 : 1) R_f (20) = 0.5, R_f (26) = 0.8). After 45 minutes the reaction mixture was diluted with dichloromethane (50ml), washed with 1M HCl (2x50ml), 1M Na_2CO_3 (50ml), water (2x50ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure. FC (160g silica, toluene : diethyl ether; 5 : 1) gave a colourless liquid (26) (1.51g, 60% yield), homogeneous by t.l.c. and ^1H n.m.r.: δ_H (400 MHz; solvent $CDCl_3$; standard Me_4Si) 0.94 (3H, t, *J* 7.5 Hz, CH_3), 1.70 (2H, sex, *J* 7.5 Hz, CH_3CH_2), 2.25 (3H, s, $C(O)CH_3$), 2.35 (2H, m, $CH_3CH_2CH_2$), 2.86 (2H, t, *J* 7 Hz, Ar-CH_2), 3.77 (1H, dd, J_{gem} -12 Hz and $J_{3,2}$ 5 Hz, CH_2OAr), 3.84 (1H, dd, J_{gem} -12 Hz and $J_{3,2}$ 5 Hz, CH_2OAr), 4.14 (1H, dd, J_{gem} -9 Hz and $J_{1,2}$ 5 Hz, CH_2Cl), 4.15 (1H, dd, J_{gem} -9 Hz and $J_{1,2}$ 5 Hz, CH_2Cl), 4.23 (2H, t, $ArCH_2CH_2$), 5.33 (1H, quintet, *J* 5 Hz, CHOC(O)), 6.89 (2H, d, J_{ortho} 9 Hz, Ar-*H*), and 7.18 (2H, d, J_{ortho} 9 Hz, Ar-*H*); δ_C (100MHz; solvent $CDCl_3$) 13.6 (C-4'), 18.4 (C-3'), 22.0 (C-11), 34.1 (C-8), 35.9 (C-2'), 42.5 (C-1), 64.9 (C-3), 65.8 (C-9), 72.0 (C-2), 114.7 (C-5), 129.6 (C-6), 130.6 (C-7), 157.1 (C-4), 170.7 (C-10), and 172.8 (C-1'); m/z (EI) 344 (M^+, 0.3%), 342 (M^+, 1), 284 ((M-acetate)$^+$, 3), 282 ((M-

acetate)$^+$, 9), and 163 (100); HRMS, found (m/z): 344.1235, $C_{17}O_5H_{23}{}^{37}Cl$, requires: 344.1204; Found (m/z): 342.1229, $C_{17}O_5H_{23}{}^{35}Cl$, requires: 342.1234.

3-[4-[2-(Hydroxy)ethyl]phenoxy]-1-chloro-2-butanoyloxypropane (27).- This compound was isolated by flash chromatography, after racemic (26) had been hydrolysed the lipase from *Mucor sp.* (lipase M); δ_H (270 MHz; solvent $CDCl_3$; standard Me_4Si) 0.95 (3H, t, *J* 7.5 Hz, CH_3), 1.70 (2H, sex, *J* 7.5 Hz, CH_3CH_2), 2.39 (2H, t, *J* 7.5 Hz, $CH_3CH_2CH_2$), 2.84 (2H, t, *J* 7 Hz, Ar-CH_2), 3.74-3.94 (4H, m, CH_2OAr and $ArCH_2CH_2$), 4.18 (2H, m, CH_2Cl), 5.37 (1H, quintet, *J* 5 Hz, CHOC(O)), 6.87 (2H, d, J_{ortho} 8.5 Hz, Ar-*H*), and 7.19 (2H, d, J_{ortho} 8.5 Hz, Ar-*H*).

Attempted hydrolase-mediated hydrolysis of (±)-3-[4-[2-acetoxyethyl] phenoxy]-1-chloro-2-butanoyloxypropane (26).- Ten enzymes were screened for the ability to hydrolyse (±)-3-[4-[2-acetoxyethyl]phenoxy]-1-chloro-2-butanoyloxypropane (26). A general protocol is: (26) (10mg, 0.03mmol), acetone (1ml), phosphate buffer pH 7, 20mM (1ml) and enzyme (*ca.* 10mg) was stirred at room temperature. Reactions were monitored directly by t.l.c. (toluene : diethyl ether; 1 : 1) R_f (26) = 0.80, R_f (21) = 0.48, R_f (27) = 0.40, R_f (24) = 0.14. The following table shows the enzymes screened, the enzyme suppliers and whether butyrate (26) was a substrate for that enzyme:

Enzyme source, code and type	Supplier	Quantity used	Hydrolysis ?
Candida cylindracea lipase	Sigma	12mg	yes
Aspergillus niger lipase A	Amano	15mg	yes
Mucor sp. lipase M	Amano	10mg	yes
Candida cylindracea lipase AY	Amano	13mg	yes
Pseudomonas sp. lipase P	Amano	15mg	yes
Rhizopus javanicus lipase N	Amano	14mg	yes
Porcine pancreatic lipase (PPL)	Sigma	12mg	yes
Pig liver esterase (PLE)	B.M.	0.5ml	yes
Orange peel acetylesterase	Sigma	0.2ml	no
Wheatgerm lipase	Sigma	0.2ml	no
Blank control		0	no

B.M. = Boeringer Mannheim

Two enzymes were selected on the grounds that the hydrolysis appeared to be regio-selective, in that only the butyrate and not the acetate function of compound (26) was being hydrolysed.

A suspension of (±)-3-[4-[2-acetoxyethyl]phenoxy]-1-chloro-2-butanoyloxypropane (26) (17mg), lipase M (12mg), Phosphate buffer 20mM, pH 7 (2ml) and acetone (0.2ml) was stirred at room temperature for 72 hours. The reaction mixture was diluted with water (10ml), extracted with diethyl ether (3x10ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure. FC (silica 2g, light petroleum ether (b.p. = 40-60 °C) : diethyl ether; 2 : 1) gave recovered starting material (26) (12.1mg, 65% yield), product (27) (1.2mg, 8% yield) and product (20) (3.0mg, 20% yield), no diol (24) was isolated. The product 3-[4-[2-acetoxyethyl]phenoxy]-1-chloropropan-2-ol (20) was converted to its corresponding Mosher's ester (23) (making sure that the reaction was complete by t.l.c., before work-up). The ee of (23) = 90±2%.

Similarly *Rhizopus javanicus* lipase-catalysed hydrolysis of (26) (19mg) gave after 72 hours, recovered starting material (26) (8.8mg, 43% yield), product (27) (2.6mg, 15% yield) and product (20) (5.9mg,36% yield), no diol (24) was isolated. The product 3-[4-[2-(acetoxy)ethyl]phenoxy]-1-chloropropan-2-ol (20) was converted to its corresponding Mosher's ester (23) (again making sure that the reaction was complete by t.l.c., before work-up). The ee of (23) ≥ 98±2%.

(±)-3-[4-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-1,2-epoxypropane (28).[104]- This compound was donated by Dr. M.B. Mitchell (SmithKline Beechams, Tonbridge). (T.l.c., (light petroleum ether (b.p. 40-60 °C) : diethyl ether; 1 : 1), R_f (28) = 0.33; ν ($CDCl_3$) 3 070 (m), 3 050 (w), 2 990 (s), 2 910 (s), 2 850 (s), 2 800 (m), 2 740 (w), 2 050 (w), 1 870 (w), 1 730 (w), 1 605 (s), 1 580 (m), 1 505 (s), 1 475 (w), 1 465 (w), 1 450 (m), and 1 425cm^{-1} (w); δ_H (220 MHz; solvent $CDCl_3$; standard Me_4Si) 0.20 (2H, m, cyclopropane methylene protons), 0.53 (2H, m, cyclopropane methylene protons), 1.06 (1H, m, cyclopropyl methine proton), 2.77 (1H, m, terminal epoxide proton), 2.87 (3H, m, ArCH_2 and terminal epoxide proton), 3.31 (2H, d, J 7.7, OCH_2-cyclopropyl protons), 3.36 (1H, m, epoxide methine proton), 3.64 (2H, t, J 9 Hz, ArCH_2CH_2), 3.97 (1H, dd, J_{gem} -11Hz and J_{anti} 5.4 Hz, ArOC*H*H (*anti*)), 4.22 (1H, J_{gem} -11Hz and J_{syn} 2.4 Hz, ArOC*H*H (*syn*)), 6.89 (2H, d, J_{ortho} 10 Hz, Ar-*H*), and 7.19 (2H, d, J_{ortho} 10 Hz, Ar-H); δ_C (100 MHz; solvent $CDCl_3$) 2.70 (C-12), 10.40 (C-11), 35.26 (C-8), 44.38 (C-1), 49.90 (C-2), 68.65 (C-3), 71.46 (C-10), 75.26 (C-9), 114.39 (C-5), 129.60 (C-6), 131.58 (C-7), and 156.79 (C-4); m/z (EI) 248 (M^+, 6%), 163 (38), 121 (3), 107 (38), 91 (11), 77 (9), 57 (35), and 55 (100); HRMS, found (m/z): 248.1404, $C_{15}H_{20}O_3$ requires: 248.1413.

(±)-3-[4-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropan-2-ol (29).- Into a solution of (±)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1,2-epoxypropane (28) (10.01g, 40mmol) in dry dichloromethane (20ml) was bubbled dry HCl gas for ten minutes. T.l.c., (light petroleum ether (b.p. 40-60 °C) : diethyl ether; 1 : 1), R_f (28) = 0.33, R_f (29) = 0.23. The solvent was evaporated under reduced pressure to give (29) (11.2g, 97% yield) as a colourless oil, homogeneous by t.l.c. and ^1H n.m.r.; ν ($CDCl_3$) 3 580 (b, OH), 3 420 (b OH), 3 000 (m), 2 930 (s), 2 850 (s), 2 740 (w), 1 605 (s), 1 580 (m), 1 505 (s), 1 235 (s),

1 170 (s), 1 080 (s), and 1 040 cm^{-1} (s); δ_H (220 MHz; solvent $CDCl_3$; standard Me_4Si) 0.19 (2H, m, cyclopropane methylene protons), 0.53 (2H, m, cyclopropane methylene protons), 1.05 (1H, m, cyclopropyl methine proton), 2.65 (1H, bm, O*H*), 2.86 (2H, t, *J* 9 Hz, ArCH_2), 3.29 (2H, d, *J* 7 Hz, OCH_2-cyclopropyl protons), 3.63 (2H, t, *J* 9Hz, ArCH_2CH_2), 3.75 (2H, m, ArOCH_2), 4.07 (2H, m, CH_2Cl), 4.22 (1H, m, *C*HOH), 6.88 (2H, d, J_{ortho} 10 Hz, Ar-*H*), and 7.19 (2H, d, J_{ortho} 10 Hz, Ar-H); δ_C (100 MHz; solvent $CDCl_3$) 2.82 (C-12), 10.45 (C-11), 35.30 (C-8), 45.82 (C-1), 68.56 (C-2), 69.77 (C-3), 71.53 (C-10), 75.43 (C-9), 114.40 (C-5), 129.78 (C-6), 131.89 (C-7), and 156.59 (C-4); m/z (EI) 286 (M^+, 13%), 284 (M^+, 38), 248 ($(M-HCl)^+$, 6), 199 (100), 107 (46), 91 (4), and 77 (4); HRMS, found (m/z): 286.1142, $C_{15}H_{21}O_3{}^{37}Cl$ requires: 286.1149; Found (m/z) : 284.1172, $C_{15}H_{21}O_3{}^{35}Cl$ requires: 284.1179.

3-[4-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropan-2-one (30).- To a cold (-2 °C) stirred solution of (±)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropan-2-ol (29) (1.97g, 6.9mmol) in AR grade acetone (100ml) was added dropwise 2.67 mol Cr^{+3} dm^{-3} Jones reagent (2.86ml, 7.6mmol) over a ten minute period The reaction was allowed to come to room temperature. After 18 hours more Jones reagent (0.76mmol, 0.1ml) was added. After a total of 19.5 hours the reaction mixture was concentrated under reduced pressure to *ca.* 5ml. T.l.c., (toluene : diethyl ether; 15 : 1), R_f (29) = 0.15, R_f (30) = 0.32. The residue was diluted with dichloromethane (80ml), washed with saturated hydrogen carbonate solution (50ml), saturated sodium chloride (2x30ml), dried ($MgSO_4$), filtered and evaporated. FC (60g silica, toluene 100%, to toluene : diethyl ether 25 : 1, to 10 : 1) yielded white lusturous crystals (30) (1.10g, 56% yield), m.p. 44-46 °C, 97% pure by ^1H n.m.r.; ν ($CDCl_3$) 2 930 (m), 2 850 (m), 1 740 (s), 1 610 (m), 1 505 (s), 1235 (br s), 1 175 (m), and 1 090 cm^{-1} (br s); δ_H (220 MHz; solvent $CDCl_3$; standard Me_4Si) 0.17 (2H, m, cyclopropane

methylene protons), 0.53 (2H, m, cyclopropane methylene protons), 1.05 (1H, m, cyclopropyl methine proton), 2.85 (2H, t, *J* 9 Hz, ArCH_2), 3.30 (2H, d, *J* 7 Hz, OCH_2-cyclopropyl protons), 3.64 (2H, t, *J* 9Hz, ArCH$_2$CH_2), 4.43 (2H, s, ArOCH_2), 4.73 (2H, s, CH_2Cl), 6.85 (2H, d, J_{ortho} 10 Hz, Ar-*H*), and 7.22 (2H, d, J_{ortho} 10 Hz, Ar-H); δ_C (100 MHz; solvent $CDCl_3$) 2.84 (C-12), 10.48 (C-11), 35.33 (C-8), 44.25 (C-1), 71.41 (C-3), 71.69 (C-10), 75.48 (C-9), 114.38 (C-5), 130.07 (C-6), 132.89 (C-7), 155.76 (C-4), and 220.48 (C-2); m/z (EI) 284 (M^+, 7%), 282 (M^+, 16), 241 (6), 211 (6), 197 (100), 161 (19), 143 (36), 133 (9), 120 (11), and 107 (60); HRMS, found (m/z): 284.1002, $C_{15}H_{19}O_3{}^{37}Cl$ requires: 284.0993; Found (m/z) : 282.1020, $C_{15}H_{19}O_3{}^{35}Cl$ requires: 284.1023.

4-[2-(Cyclopropylmethoxy)ethyl]phenol (31).- Phenol (31) was donated by Dr M.B. Mitchell (SmithKline Beechams Ltd, Tonbridge). It is a colourless liquid. T.l.c., (toluene : diethyl ether; 15 : 1), R_f (31) = 0.19; (light petroleum ether (b.p. = 40-60 °C) : ethyl acetate; 1 : 1), R_f (31) = 0.32; ν ($CDCl_3$) 3 580 (OH), 3 300 (bs), 3 070 (m), 3 000 (m), 2 930 (s), 2 850 (s), 1 610 (s), 1 505 (s), 1 220 (bs), 1 165 (s), and 1 095 cm^{-1} (s); δ_H (220 MHz; solvent $CDCl_3$; standard Me_4Si) 0.19 (2H, m, cyclopropane methylene protons), 0.55 (2H, m, cyclopropane methylene protons), 1.05 (1H, m, cyclopropyl methine proton), 2.95 (2H, t, *J* 9 Hz, ArCH_2), 3.31 (2H, d, *J* 7 Hz, OCH_2-cyclopropyl protons), 3.65 (2H, t, *J* 9Hz, ArCH$_2$CH_2), 5.50 (1H, bs, CHO*H*, exchangable with D_2O), 6.87 (2H, d, J_{ortho} 10 Hz, Ar-*H*), and 7.07 (2H, d, J_{ortho} 10 Hz, Ar-H); δ_C (100 MHz; solvent $CDCl_3$) 2.94 (C-9), 10.32 (C-8), 35.10 (C-5), 71.71 (C-7), 75.58 (C-6), 115.20 (C-2), 129.71 (C-3), 130.17 (C-3), and 154.27 (C-1); m/z (EI) 192 (M^+, 24%), 121 (18), 120 (17), 107 (100), 103 (6), 91 (11), 77 (22), and 55 (74); HRMS, found (m/z) : 192.1148, $C_{11}H_{16}O_2$ requires: 192.1150.

(2(S,R),2'(R))-3-[4-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-1-chloro-2-(R,S)-[(R)-2'-phenyl-2'-[acetoxy]acetoxy]propane (32).[106,107]- To a stirred

cold (-11 °C) solution of (R)-(-)-*O*-acetylmandelic acid (0.75g, 0.39mmol), 4-dimethylaminopyridine (cat.), in dichloromethane (1ml) was added (±)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropan-2-ol (29) (0.100g, 0.35mmol dissolved in dicloromethane (0.5ml)). To this colourless solution was added 1,3-dicyclohexylcarbodiimide (0.080g, 0.38mmol). An immediate precipitate of 1,3-dicyclohexylurea was observed. T.l.c., (toluene : diethyl ether; 10 : 1), R_f (29) = 0.25, R_f (31) = 0.52. After 195 min the reaction mixture was filtered through glass wool. FC (silica 12g, light petroleum ether (b.p. 40-60 °C) : diethyl ether; 3 : 1) yielded (31) as a colourless oil (0.152g, 94% yield), homogeneous by ^{1}H n.m.r. and t.l.c.; ν ($CDCl_3$) 2 920 (m), 2 850 (m), 1 740 (s), 1 605 (m), 1 580 (w), 1 505 (s), 1 450 (m), 1 230 (s), 1 200 (m), 1 170 (s), 1 080 (s), and 1 040 cm^{-1} (s); δ_H (400 MHz; solvent $CDCl_3$; standard Me_4Si) 0.19 (2H, m, cyclopropane methylene protons), 0.53 (2H, m, cyclopropane methylene protons), 1.04 (1H, m, cyclopropyl methine proton), 2.18 (1.5H, s, C(O)C*H*$_3$, (2R,2'R)-diastereomer), 2.20 (1.5H, s, C(O)C*H*$_3$, (2S,2'R)-diastereomer) 2.83 (2H, t+t, *J* 9 Hz, ArC*H*$_2$), 3.27 (2H, d, *J* 6.9 Hz, OC*H*$_2$-cyclopropyl protons), 3.60 (2H, t+t, *J* 9Hz, ArCH$_2$C*H*$_2$), 3.75 (2H, m, ArOC*H*$_2$), 4.07 (2H, m, C*H*$_2$Cl), 5.33 (1H, m, C*H*CH$_2$Cl), 5.91 (0.5H, s, ArC*H*, (2R,2'R)-diastereomer), 5.92 (0.5H, s, ArC*H*, (2S,2'R)-diastereomer), 6.67-6.81 (2H, m, Ar-*H*), 7.08-7.15 (2H, m, Ar-H), 7.37 (3H, m, Ar-*H*), and 7.47 (2H, m, Ar-*H*); δ_C (100 MHz; solvent $CDCl_3$) 2.77 (C-12), 10.44 (C-11), 20.36 (C-2"), 35.29 (C-8), 41.72 + 41.92 (C-1), 65.63 + 65.69 (C-2), 71.47 (C-10), 72.07 + 71.97 (C-3), 74.35.+ 74.42 (C-2'), 75.33 (C-9), 114.43 + 114.50 (C-5), 127.36 + 127.45 (C-6'), 128.58 (C-5'), 129.09 + 129.14 (C-4'), 129.62 + 129.71 (C-6), 131.89 + 131.97 (C-7),133.07 (C-3'), 156.38 + 156.41 (C-4), 167.92 + 167.96 (C-1'), and 170.08 (C-1"); m/z (EI) 462 (M$^+$, 8%), 460 (M$^+$, 25), 269 (100), 210 (21), 147 (10), 135 (7), 121 (14), 107 (52), 91 (16), and 77 (12) HRMS, found (m/z): 462.1641, $C_{25}H_{29}O_6{}^{37}Cl$ requires: 462.1623; Found: 460.1655, $C_{25}H_{29}O_6{}^{35}Cl$ requires: 460.1653.

The ^1H n.m.r. data for the pure diastereomers (32) of (R)-(-)-*O*-acetylmandelic ester of alcohol (29) (obtained by enzymatic resolution of alcohol(29)) is given below;

(2(S),2'(R))-3-[4-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-1-chloro-2-(S)-[(R)-2'-phenyl-2'-(acetoxy)acetoxy]propane (32).- δ_H (400 MHz; solvent $CDCl_3$; standard Me_4Si) 0.19 (2H, m, cyclopropane methylene protons), 0.53 (2H, m, cyclopropane methylene protons), 1.04 (1H, m, cyclopropyl methine proton), 2.18 (3H, s, C(O)CH_3), 2.83 (2H, t, *J* 9 Hz, ArCH_2), 3.27 (2H, d, *J* 6.9 Hz, OCH_2-cyclopropyl protons), 3.59 (2H, t, *J* 9Hz, ArCH_2CH_2), 3.81 (2H, m, ArOCH_2), 4.02 (2H, m, CH_2Cl), 5.33 (1H, m, CHCH$_2$Cl), 5.92 (1H, s, ArCH), 6.68 (2H, J_{ortho} 8.6 Hz, Ar-*H*), 7.09 (2H, J_{ortho} 8.6 Hz, Ar-*H*), 7.37 (3H, m, Ar-*H*), and 7.47 (2H, m, Ar-*H*);

(2(R),2'(R))-3-[4-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-1-chloro-2-(R)-[(R)-2'-phenyl-2'-(acetoxy)acetoxy]propane (32).- δ_H (400 MHz; solvent $CDCl_3$; standard Me_4Si) 0.19 (2H, m, cyclopropane methylene protons), 0.53 (2H, m, cyclopropane methylene protons), 1.04 (1H, m, cyclopropyl methine proton), 2.20 (3H, s, C(O)CH_3), 2.84 (2H, t, *J* 9 Hz, ArCH_2), 3.27 (2H, d, *J* 6.9 Hz, OCH_2-cyclopropyl protons), 3.60 (2H, t, *J* 9Hz, ArCH_2CH_2), 3.68 (2H, m, ArOCH_2), 4.15 (2H, m, CH_2Cl), 5.33 (1H, m, CHCH$_2$Cl), 5.91 (1H, s, ArCH), 6.86 (2H, J_{ortho} 8.6 Hz, Ar-*H*), 7.14 (2H, J_{ortho} 8.6 Hz, Ar-*H*), 7.37 (3H, m, Ar-*H*), and 7.47 (2H, m, Ar-*H*).

Baker's yeast reduction of 3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropan-2-one (30).- To warm distilled water (y = 1000x ml) was added sucrose or glucose (0.02y g), if allyl alcohol (xg) was used in the reaction this was also added at this stage and fresh baker's yeast (Sainsbury's)

(0.02*y* g). The suspension was stirred for about 30 minutes. Then 3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropan-2-one (30) (*x*g; 1g/l or 0.33*x* g; 3g/l) either neat or pre-dissolved in absolute ethanol (20xml, 2%v/v). The reaction was monitored by t.l.c., toluene : diethyl ether; 10 : 1, R_f (30) = 0.51, R_f (29) = 0.22. The reaction mixture was worked-up. The suspension was centrifuged (5,000 r.p.m. / 10 mins). The supernatant was removed. The pellet was resuspended in water (50ml) and centrifuged (5,000 r.p.m. / 10mins). The combined supernatants were extracted with ether (2x100ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure. FC (silica 16g, light petroleum ether (b.p. 40-60 °C) : diethyl ether; 9 : 1, to 4 : 1, to 1 : 1), afforded product (29) as an oil. The ee was determined by synthesising the (R)-*O*-acetylmandelic ester (32) as described above (making sure the reactions were complete by t.l.c. before work-up). The full results are:

Conditions	Yield / %	ee / %	R/S
Baker's yeast, 72 hours, (30) neat (*x* = 0.02, 3mg/ml).	39	42	R
Baker's yeast, 24 hours, (30) dissolved in ethanol (*x* = 0.033, 3mg/ml), sucrose.	24	42	R
Baker's yeast, 19 hours, (30) dissolved in ethanol (*x* = 0.100, 1mg/ml), sucrose.	63	50	R
Baker's yeast, 24 hours, (30) neat (*x* = 0.025, 1mg/ml), glucose.	59	47	R
Baker's yeast, 48 hours, (30) neat (*x* = 0.025, 1mg/ml), glucose, allyl alcohol (lit., ref 53)	38	52	R

Laboratory yeast strains screened for their ability to reduce 3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropan-2-one (30).- Ten yeast strains were screened. The yeasts were stored at 4 °C on YM agar slopes. YM broth was obtained from Difco laboratories, Detroit, Michigan,

U.S.A. "Ogihara 1" was donated by Professor Y. Ogihara, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, City University, Nagoya, Japan, and is Oriental Baker's Yeast (Oriental Yeast Co. Ltd, Japan). "OY" yeasts were isolated from Oriental Yeast and were donated by Enzymatics, U.K. BLY is Burton's larger yeast. NCYC yeasts were obtained from the National Collection of Yeast Cultures, Norwich, U.K.

All operations were done under sterile conditions. A 25ml conical flask containing YM media (10ml) was innoculated with a loopful of culture, and shaken at 30 °C for 24 hours. Then a one in ten dilution was performed to fresh YM media (50ml). After 24 hours 3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropan-2-one (30) (50mg, 1g/L; dissolved in ethanol (0.5ml) 1% v/v) was added. The reactions were monitored by t.l.c., (toluene : diethyl ether; 10 : 1), R_f (30) = 0.51, R_f (31) = 0.27, R_f (29) = 0.22. The reactions which produced the most amount of alcohol product 3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropan-2-ol (29) were worked-up. The suspension was centrifuged (5,000 r.p.m. / 10 mins). The supernatant was removed, and the pellet was resuspended in water (50ml) and centrifuged (5,000 r.p.m. / 10mins). The combined supernatants were extracted with ether (2x100ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure. FC (silica 16g, light petroleum ether (b.p. 40-60 °C) : diethyl ether; 9 : 1, to 4 : 1, to 1 : 1), afforded product (29) as an oil. The %ee was determined by synthesising the (R)-*O*-acetylmandelic ester (32) as described above (making sure that the reactions were complete by t.l.c. before the reactions were worked-up). The results are given Table 2.5, Section 2.7. The following yeasts were not worked-up, *Saccharomyces cerevisiae* NCYC 1765, OY P2, OY R1, OY R2, BLY B, because the reduction of (30) was very slow. The major product in these cases was 4-[2-(cyclopropylmethoxy)ethyl]phenol (31), which was

identified by t.l.c. and ^1H n.m.r.

Other microbiologically mediated reduction of 3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropan-2-one (30).- These reduction experiments were performed by E. Tidswell (Department of Microbiology and Botany, University College of Wales, Aberystwyth). To a growing culture of microorganism was added 3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropan-2-one (30) (1mg/ml, 0.050g). The reduction was monitored by t.l.c., (toluene : diethyl ether; 10 : 1), R_f (30) = 0.51, R_f (31) = 0.27, R_f (29) = 0.22. After work-up the desired product 3-[4-[2-(cyclopropylmetyl)ethyl]phenoxy]-1-chloropropan-2-ol (29) and the phenol breakdown product (31) were separated by flash chromatography. FC (6g silica, (light petroleum ether (b.p. 40-60 °C) : diethyl ether; 4 : 1, to 2 : 1). The alcohol (29) was converted into the (R)-*O*-acetylmandelic ester (32), as described above (making sure that the reactions were complete by t.l.c. before the reactions were worked-up). The full results are given below:

microorganism	Carbon growth source	(31) /y%	(29) /y%	(29) /ee%	(29) R/S
Clostridium pasteuranium 6013	Glucose	n.d.	9	67	R
C.tyrobutyricum LA1 (Aux P)	Glucose	n.d.	3	27	R
C.tyrobutyricum LA1 (Aux P)	Crotonate	70	8	44	R
mutant of Aux P (TM V)	glucose	47	3	39	R
mutant of Aux P (TM V)	crotonate	46	0	-	-
Lactobacillus brevis	fructose	16	32	28	S

(±)-3-[4-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-1-chloro-2-acetoxypropane (33).- To an ice cold solution of (±)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropan-2-ol (29) (3.09g, 11mmol) in dry dichloromethane (20ml) and dry pyridine (10ml), was added acetic anhydride (10ml, 105mmol). The reaction mixture

was allowed to slowly come to room temperature. T.l.c., (light petroleum ether (b.p. 40-60 °C) : diethyl ether; 1 : 1), R_f (29) = 0.23, R_f (33) = 0.53. After 14 hours the reaction mixture was diluted with diethyl ether (100ml), washed with 1M HCl (50ml), saturated $NaHCO_3$ solution (2x50ml) (VIGOROUS !), saturated sodium chloride (50ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure, to yield a colourless liquid (33) (3.08g, 87% yield), homogeneous by t.l.c. and ^1H n.m.r.; ν ($CDCl_3$) 3 000 (w), 2 930 (m), 2 850 (s), 2 700 (w), 1 735 (s), 1 605 (m), 1 580 (m), 1 505 (s), 1 365 (m), 1 225 (s), 1170 (m), 1 085 (s), and 1 040 cm^{-1} (s); δ_H (220 MHz; solvent $CDCl_3$; standard Me_4Si) 0.20 (2H, m, cyclopropane methylene protons), 0.55 (2H, m, cyclopropane methylene protons), 1.06 (1H, m, cyclopropyl methine proton), 2.13 (3H, s, C(O)C*H*$_3$), 2.87 (2H, d, *J* 9 Hz, ArC*H*$_2$), 3.30 (2H, d, *J* 7 Hz, OC*H*$_2$-cyclopropyl protons), 3.63 (2H, t, *J* 9Hz, ArCH$_2$C*H*$_2$), 3.84 (2H, m, ArOC*H*$_2$), 4.17 (2H, m, C*H*$_2$Cl), 5.35 (1H, m, C*H*OAc), 6.89 (2H, d, J_{ortho} 10 Hz, Ar-*H*), and 7.20 (2H, d, J_{ortho} 10 Hz, Ar-H); δ_C (100 MHz; solvent $CDCl_3$) 2.78 (C-12), 10.46 (C-11), 20.65 (C-2'), 35.32 (C-8), 45.34 (C-1), 66.02 (C-10), 71.04 (C-3), 71.52 (C-2), 75.38 (C-9), 114.46 (C-5), 129.75 (C-6), 131.96 (C-7), 156.59 (C-4), and 169.81 (C-1'); m/z (EI) 328 (M^+, 1%), 326 (M^+, 3), 137 (36), 135 (100), 107 (10), 91 (6), and 77 (5) HRMS, found (m/z): 328.1258, $C_{17}H_{23}O_4{}^{37}Cl$, requires: 328.1256; Found (m/z): 326.1281, $C_{15}H_{21}O_3{}^{35}Cl$ requires: 326.1285.

Initial enzyme screening experiment, for the ability of an enzyme to resolve racemic 3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloro-2-acetoxypropane (33).- Ten enzymes were screened for their ability to hydrolyse (33). A suspension of (±)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloro-2-acetoxypropane (33) (20mg), phosphate buffer, pH 7, 100mM (1ml) and enzyme (*ca.* 10mg) was vigorously stirred at room temperature. The

reactions were monitored by t.l.c., (light petroleum ether (b.p. 40-60 °C) : diethyl ether; 1 : 1), R_f (33) = 0.53, R_f (29) = 0.23. Reactions that appeared to terminate at 50% completion were diluted with water (5ml) and extracted with diethyl ether (3x10ml), dried ($MgSO_4$) filtered and evaporated under reduced pressure. FC (silica 14g, light petroleum ether (b.p. 40-60 °C) : diethyl ether; 4 : 1) to give starting material (33) and product alcohol (29). The alcohol (29) was converted into its corresponding (R)-*O*-acetylmandelic ester (32). The residual starting material (33) was hydrolysed chemical. A typical procedure is as follows. A cold (-11 °C) suspension of 3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloro-2-acetoxypropane (33) (0.111g, 0.34mmol) and anhydrous potassium carbonate (0.094g, 0.68mmol) in anhydrous methanol (4ml) was stirred for 30 minutes. T.l.c., (light petroleum ether (b.p. 40-60 °C) : diethyl ether; 1 : 1), R_f (33) = 0.53, R_f (29) = 0.23. The reaction mixture was diluted with water (20ml) and extracted with diethyl ether (2x25ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure to yield a colourless liquid (29) (0.092g, 96% yield), homogeneous by t.l.c. and ^1H n.m.r. The alcohol (29) was then converted into its corresponding (R)-*O*-acetyl mandelic ester (32) (making sure that the reaction was complete by t.l.c.). The results are as follows:

Enzyme, source, code and supplier	Time /days	SM(33) %ee,AC	P(29) %ee,AC	c	E	enzyme quantity
lipase, *Candida cylindracea*, Sigma	-	n.d.	n.d.	n.d.	n.d.	11mg
lipase A, *Aspergillus niger*, Amano	4	73,S	29,R	0.72	4	12mg
lipase P, *Pseudomonas sp.*, Amano	-	n.d.	n.d.	n.d.	n.d.	14mg
lipase F-AP15, *Rhizopus sp.*, Amano	4	10,R	28,S	0.26	2	10mg
lipase M, *Mucor sp.*, Amano	4	25,R	54,S	0.32	4	11mg
lipase N, *Rhizopus sp.*, Amano	4	18,R	27,S	0.40	2	15mg
Porcine pancreatic lipase, Sigma	-	36,R	54,S	0.40	5	11mg
lipase *Geotrichium candidum*, (B)	4	n.d.	n.d.	n.d.	n.d.	10mg
lipase *Pseudomonas fluorescens*, (B)	4	>97,R	57,S	0.63	14	10mg
PLE, Boehringer Mannheim	-	2,R	5,S	0.29	1	10µl
blank (control)		-	-	-	-	-

(B) = Biocatalysis Ltd.; AC = absolute configuration.

Scale-up of the lipase *ex Pseudomonas fluorescens* catalysed resolution of racemic 3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloro-2-acetoxypropane (33).- A suspension of (±)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloro-2-acetoxypropane (33) (1.00g), phosphate buffer, pH 7, 100mM (100ml) and lipase from *Pseudomonas fluorescens* (Biocatalysis Ltd.) (0.469g) was vigorously stirred at room temperature for 3days. The usual determination of the E value as described above gave (R)-(33) (0.193g, 19% yield, 90%ee) and (S)-(29) (0.598g, 68% yield, 36%ee), c = 0.714, E = 6.

(±)-3-[4-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-1-chloro-2-butanoyloxypropane (34).- To an ice cold solution, under an argon atmosphere, of (±)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropan-2-ol (29) (0.415g, 1.46mmol) in dry dichloromethane (10ml) and dry pyridine (2ml), was added 4-dimethylaminopyridine (cat.) and distilled butanoic anhydride (0.36ml, 2.2mmol). The reaction was allowed to slowly come to room temperature, t.l.c., (light petroleum ether (b.p. 40-60 °C) : diethyl ether; 1 : 1), Rf (29) = 0.23, Rf (34) = 0.84, after 15 hours the reaction mixture was diluted with with dichloromethane (20ml), washed with 1M HCl (10ml), saturated sodium hydrogen carbonate (20ml), water (10ml), dried ($MgSO_4$), filtered and then evaporated under reduced pressure to yield (34) as a colourless oil (0.465g, 90% yield), homogeneous by t.l.c. and ^{1}H n.m.r.; ν ($CDCl_3$) 2 960 (w), 2 930 (m), 2 860 (m), 1 730 (s), 1 650 (s), 1 580 (w), 1 505 (s), 1 235 (s), 1 170 (s), and 1 085 cm^{-1} (s); δ_H (220 MHz; solvent $CDCl_3$; standard Me_4Si) 0.18 (2H, m, cyclopropane methylene protons), 0.53 (2H, m, cyclopropane methylene protons), 0.95 (5H, m, CH_3 and cyclopropyl methine proton), 1.67 (2H, m, $C(O)CH_2CH_2CH_3$), 2.37 (2H, t, *J* 10 Hz, $C(O)CH_2$), 2.87 (2H, d, *J* 9 Hz, $ArCH_2$), 3.30 (2H, d, *J* 7 Hz, OCH_2-

cyclopropyl protons), 3.63 (2H, t, J 9Hz, $ArCH_2CH_2$), 3.85 (2H, m, $ArOCH_2$), 4.37 (2H, m, CH_2Cl), 5.38 (1H, m, CH), 6.91 (2H, d, J_{ortho} 10 Hz, Ar-H), and 7.23 (2H, d, J_{ortho} 10 Hz, Ar-H); δ_C (100 MHz; solvent $CDCl_3$) 2.77 (C-12), 10.45 (C-11), 13.37 (C-4'), 18.24 (C-3'), 35.32 (C-8), 35.92 (C-2'), 42.47 (C-1), 66.12 (C-10), 70.76 (C-3), 71.53 (C-2), 75.39 (C-9), 114.48 (C-5), 129.73 (C-6), 131.94 (C-7), 156.61 (C-4), and 172.59 (C-1'); m/z (EI) 354 (M^+, 1%), 354 (M^+, 3), 163 (100), 107 (6), 91 (6), 77 (4), 55 (72), and 43 (38); HRMS, found (m/z): 356.1569, $C_{19}H_{27}O_4{}^{37}Cl$ requires: 356.1568; Found (m/z): 354.1598, $C_{19}H_{27}O_4{}^{35}Cl$ requires: 354.1598.

Enzymatic hydrolysis of the butyrate (±)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloro-2-butanoyloxypropane (34).- A suspension of (±)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloro-2-butanoyloxypropane (34) (*ca* 0.06g), phosphate buffer, pH 7, 100mM (3ml) and enzyme (ca. 0.03g) was vigorously stirred at room temperature for 14 hours. E value determined as usual.

lipase from	enzyme /mg	sm (33) /mg	residual (33) %y,%ee,AC	product (29) %y,%ee,AC	c	E
Pseudomonas fluorescens	31	65	43,99,R	59,75,S	0.57	35
Lipase M (Amano)	37	62	65,15,R	20,82,S	0.15	10
Lipase N (Amano)	32	60	70,10,R	34,77,S	0.11	10

Scale-up of the lipase *ex Pseudomonas fluorescens* catalysed resolution of racemic 3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloro-2-butanoyloxypropane (34).- A suspension of (±)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloro-2-butanoyloxypropane (34) (0.20g), phosphate buffer, pH 7, 100mM (10ml)

and lipase *ex Pseudomonas fluorescens* (Biocatalysts, Ltd.) (0.10g) was vigorously stirred at room temperature for 14 hours. The usual determination of the E value as described above gave (R)-(-)-(34) (0.089g, 44% yield, 95%ee $[\alpha]_D$ = -21.6 ° (c 0.89 $CHCl_3$)) and (+)-(S)-(29) (0.092g, 56% yield, 82%ee, $[\alpha]_D$ = +0.9 (c 0.76 $CHCl_3$)), c = 0.537, E = 37.

Conversion of the residual starting material (R)-(-)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloro-2-butanoyloxypropane (34) (95%ee) to (R)-(+)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1,2-epoxypropane (28), And subsequent conversion to (S)-(-)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-2-hydroxypropylamine (37).- To a stirred solution, under argon, of (R)-(-)-3-[4-[2-(cyclopropylmethoxy) ethyl]phenoxy]-1-chloro-2-butanoyloxypropane (34) (95%ee) (0.063g, 0.18mmol) and freshly made potassium *tert*-butoxide (0.024g, 0.21mmol) in dry *tert*-butanol (2ml) was heated at 60 °C for two hours, t.l.c., (light petroleum ether (b.p. 40-60 °C) : diethyl ether; 1 : 1), Rf (34) = 0.58, Rf (28).= 0.50. The reaction mixture was cooled, and diluted with water (10ml). The solution was extracted with diethyl ether (3x20ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure to yield (R)-(+)-(28) (0.041g, 93% yield, $[\alpha]_D$ = +2.02 ° (c 0.816, $CHCl_3$)) homogeneous by t.l.c. and ^1H n.m.r. The ^1H n.m.r. and t.l.c. are identical to racemic (28). The epoxide was used directly for the next step. To a stirred solution of (R)-(+)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1,2-epoxypropane (28) (0.041g, 0.17mmol) in anhydrous methanol (1.5ml) was added 0.88 S.G. concentrated ammonia (2ml). T.l.c., (dichloromethane : methanol; 5 : 1), R_f (28) = 0.87, R_f (37) = 0.12. After 18 hours the solvents were evaporated at reduced pressure. FC (silica 16g, dichloromethane : methanol; 4 : 1) gave a white solid (S)-(-)-(37) (0.026g, 60% yield, $[\alpha]_D$ = -14.11 ° (c 1.32, EtOH:H_2O:concHCl; 17:2:1, Lit.,[104] $[\alpha]_D$ = -17.77 ° (c 1.1, EtOH:H_2O:concHCl;

17:2:1)), homogeneous by t.l.c and ^1H n.m.r. and identical to previously synthesised racemic (37). (For analytical data see synthesis of racemic (37) below). ^1H n.m.r. data equivalent to the Lit.,[104] ^1H n.m.r. data.

(±)-3-[4-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-1-chloro-2-hexanoyloxypropane (34).[108] To an ice cold solution, under an argon atmosphere, of (±)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropan-2-ol (29) (0.406g, 1.4mmol) in dry dichloromethane (10ml), was added 4-dimethylaminopyridine (cat.) and hexanoic acid (0.22ml, 1.7mmol) and stirred for twenty minutes. Then 1,3-dicyclocarbodiimide (0.33g, 1.57mmol was added). The reaction mixture was allowed to slowly come to room temperature. T.l.c., (light petroleum ether (b.p. 40-60 °C) : diethyl ether; 1 : 1), R_f (29) = 0.23, R_f (35) = 0.90. After 14 hours the suspension was filtered through glass wool and evaporated under reduced pressure. FC (silica 34g, (light petroleum ether (b.p. 40-60 °C) : diethyl ether; 8 : 1) gave a colourless liquid (35) (0.463g, 85% yield), homogeneous by t.l.c. and ^1H n.m.r.; ν ($CDCl_3$) 2 960 (s), 2 920 (s), 2 860 (s), 1 730 (s), 1 605 (m), 1 505 (s), 1 455 (m), 1 235 (s), 1 160 (s), and 1 085 cm^{-1} (s); δ_H (220 MHz; solvent $CDCl_3$; standard Me_4Si) 0.19 (2H, m, cyclopropane methylene protons), 0.53 (2H, m, cyclopropane methylene protons), 0.89 (3H, t, *J* 9 Hz, CH_3), 1.06 (2H, m, cyclopropyl methine proton), 1.32 (4H, m, $CH_2CH_2CH_3$), 1.65 (2H, m, $C(O)CH_2CH_2$), 2.38 (2H, t, *J* 10 Hz, $C(O)CH_2$), 2.87 (2H, d, *J* 9 Hz, $ArCH_2$), 3.32 (2H, d, *J* 7 Hz, OCH_2-cyclopropyl protons), 3.65 (2H, t, *J* 9Hz, $ArCH_2CH_2$), 3.86 (2H, m, $ArOCH_2$), 4.19 (2H, m, CH_2Cl), 5.48 (1H, m, CH), 6.97 (2H, d, J_{ortho} 10 Hz, Ar-*H*), and 7.23 (2H, d, J_{ortho} 10 Hz, Ar-H); δ_C (100 MHz; solvent $CDCl_3$) 2.82 (C-12), 10.50 (C-11), 13.69 (C-6'), 22.12 (C-5'), 24.46 (C-4'), 31.07 (C-3'), 34.06 (C-2'), 35.38 (C-8), 42.50 (C-1), 66.15 (C-10), 70.82 (C-3), 71.58 (C-2), 75.43 (C-9), 114.52 (C-5), 129.78 (C-6), 131.98 (C-7), 156.66 (C-4), and 172.82 (C-1'); m/z (EI) 284 (M^+, 1%), 282 (M^+, 3), 193 (35), 191 (100), 99

(16), 77 (17), 55 (66), and 43 (24); HRMS, found (m/z): 384.1889, $C_{21}H_{31}O_4{}^{37}Cl$ requires: 384.1883; Found (m/z) 382.1913, $C_{21}H_{31}O_4{}^{35}Cl$ requires: 382.1911.

Lipase *ex Pseudomonas fluorescens* catalysed resolution of racemic 3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloro-2-hexanoyloxypropane (35).- A suspension of (±)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloro-2-hexanoyloxypropane (35) (0.065g), phosphate buffer, pH 7, 100mM (3ml) and lipase *ex Pseudomonas fluorescens* (Biocatalysts, Ltd.) (0.033g) was vigorously stirred at room temperature for 14 hours. The usual determination of the E value as described above gave (R)-(35) (0.029g, 46% yield, 80%ee), and (S)-(+)-(29) (0.034g, 71% yield, 83%ee), c = 0.49, E = 27.

Lipase from *Pseudomonas fluorescens* (Biocatalysts Ltd) catalysed esterification of racemic 3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropan-2-ol (29) under potentially reversible conditions.- A typical procedure: Into a 2 dram sample vial was placed a suspension of lipase from *Pseudomonas fluorescens* (Biocatalysts Ltd) (*ca.* 16mg), 3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropan-2-ol (29) (*ca.* 51mg), isooctane (Gold label, 2ml) and carboxylic acid or anhydride (3-10 mol eq.). A stirring bar was added, the vial was sealed and shaken on a rotary orbital shaker at 55 °C. (Control experiments were carried out at half the above scale). The reactions were monitored by t.l.c., (light petroleum ether (b.p. 40-60 °C) : diethyl ether; 1 : 1), R_f (29) = 0.23. Reaction in which enzyme-catalysed esterification took place were worked-up. The reaction mixture was filtered through glass wool and washed through with dichloromethane. The filtrate was evaporated under reduced pressure. FC (silica 12g, light petroleum ether (b.p. 40-60 °C) : diethyl ether 4 : 1, to 2 :

1). The E value was established in the usual manner. The results are as follows:

acyl donor	lipase /mg	acylation ?	sm (29) %ee,AC	product %ee,AC	c	E	Time /hrs
Acetic acid,100µl,9.7mol eq.	19	no	n.d.	n.d.	-	-	-
Acetic acid,50µl,9.7mol eq.	0	no					
Acetic anhydride,100µl,3.2moleq.	16	yes	n.d.	n.d.	-	-	-
Acetic anhydride,50µl,3.2mol eq.	0	yes					
Butanoic acid,100µl,5.7mol eq.	16	yes	5,R	98,S	0.05	104	8.5
Butanoic acid,50µl,5.7mol eq.	0	no					
Hexanoic acid,100µl,4.5mol eq.	16	yes	13,R	97,S	0.12	74	24
Hexanoic acid,50µl,4.5mol eq.	0	no					

Lipase from *Pseudomonas fluorescens* (Biocatalysts Ltd) catalysed acetylation of racemic 3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropan-2-ol (29) under irreversible conditions.- Into a sample vial was placed lipase from *Pseudomonas fluorescens* (Biocatalysts Ltd) (16mg), dry, distilled solvent (1ml), racemic 3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropan-2-ol (29) (*ca*. 50mg) and the acyl donor (5-1000 mol eq.), (control experiments were undertaken at half the above scale). The suspension was stirred at room temperature. T.l.c., (light petroleum ether (b.p. 40-60 °C) : diethyl ether; 1 : 1), R_f (29) = 0.23, R_f (33) = 0.53. The reactions were worked-up by filtration, the filtrate was evaporated under reduced pressure. FC (silica 14g, light petroleum ether (b.p. 40-60 °C) : diethyl ether; 2 : 1). The E value was determined in the usual manner.

(29) /mg	acyl donor	solvent /ml	Time /hrs	recovered sm (29) %y,%ee,AC	product (33) %y,%ee,AC	c	E
51	Isopropenyl acetate,100µl,5mol.eq.	THF	48	75,28,R	27,98,S	0.22	130
26	Isopropenyl acetate,50µl,5mol.eq.	THF	-	-	-	-	-
46	Isopropenyl acetate,100µl,6mol.eq.	CH_2Cl_2	slow	n.d.	n.d.	n.d.	n.d.
23	Isopropenyl acetate,50µl,6mol.eq.	CH_2Cl_2	-	-	-	-	-
51	Vinyl acetate,1000µl,61mol eq.	-	24	62,48,R	33,>98,S	0.33	159
25	Vinyl acetate,500µl,61mol eq.	-	-	-	-	-	-

Scale-up of the Lipase from *Pseudomonas fluorescens* (Biocatalysts Ltd) catalysed acetylation of racemic 3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropan-2-ol (29) under irreversible conditions, using vinyl acetate.- A suspension of lipase from *Pseudomonas fluorescens* (Biocatalysts Ltd) (0.306g), racemic 3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropan-2-ol (29) (0.962g) and vinyl acetate (20ml) was stirred at room temperature for 65 hours, filtered and evaporated. FC and determination of the E value in the usual manner gave: (R)-(-)-(29) (0.453g, 47% yield, 88%ee $[\alpha]_D$ = -0.45 °(C 5.88, $CHCl_3$)) and (S)-(+)-(33) (0.521g, >98%ee, 47% yield, $[\alpha]_D$ = +23.6 °(C 1.19, $CHCl_3$)); c = 0.471, E = 502. The residual starting material (R)-(-)-(29) (88%ee) (0.395g), *Pseudomonas fluorescens* (Biocatalysis Ltd) (0.30g) and vinyl acetate (10ml) was stirred at room temperature for 4 days, filtered and evaporated under reduced pressure FC (silica 15g, light petroleum ether (b.p. = 40-60 °C) : diethyl ether; 4 : 1, to 2 : 1) gave (R)-(-)-(29) (0.345g, 87% yield, >98%ee) and (33) (0.036g, 8% yield).

Conversion of the product (S)-(+)-3-[4-[2-(cyclopropylmethoxy) ethyl]phenoxy]-1-chloro-2-acetoxypropane (33) (>98%ee) to (S)-(-)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1,2-epoxypropane (28). And subsequent conversion to (R)-(+)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-2-hydroxypropylamine (37).- To a stirred solution, under argon, of (S)-(+)-3-[4-[2-(cyclopropylmethoxy) ethyl]phenoxy]-1-chloro-2-acetoxypropane (34) (>98%ee) (0.303g, 9.3mmol) and freshly made potassium *tert*-butoxide (0.125g, 1.11mmol) in dry *tert*-butanol (5ml) was heated at 60 °C for three hours. *Tert*-butoxide (0.020g, 0.18mmol) was added and the solution heated at 60 °C for a further 3 hours, t.l.c., (light petroleum ether (b.p. 40-60 °C) : diethyl ether; 1 : 1), R_f

(33) = 0.58, R_f (28) = 0.50. The reaction mixture was cooled diluted with water (15ml), extracted with diethyl ether (3x20ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure to yield (S)-(-)-(28) (0.219g, 95% yield, $[\alpha]_D$ = -2.56 ° (c 5.91, $CHCl_3$)) homogeneous by t.l.c. and ^1H n.m.r. The ^1H n.m.r. and t.l.c. are identical to racemic (28). The epoxide was used directly for the next step. To a stirred solution of (S)-(-)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1,2-epoxypropane (28) (0.219g, 0.88mmol) in anhydrous methanol (1.5ml) was added 0.88 S.G. concentrated ammonia (2ml). T.l.c., (dichloromethane : methanol; 5 : 1), R_f (28) = 0.87, R_f (37) = 0.12. After 23 hours the solvents were evaporated under reduced pressure. FC (silica 14g, dichloromethane : methanol; 4 : 1) gave a white solid (R)-(+)-(37) (0.134g, 57% yield, $[\alpha]_D$ = +18.06 ° (c 1.02, EtOH:H_2O:concHCl; 17:2:1, Lit.[104], $[\alpha]_D$ = +17.9 ° (c -1.1, EtOH:H_2O:concHCl; 17:2:1)), homogeneous by t.l.c and ^1H n.m.r. and identical to previously synthesised racemic (37) (for analytical data see synthesis of racemic (37) below). ^1H n.m.r. data equivalent to the Lit.,[104] ^1H n.m.r. data.

(±)-3-[4-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-1-methoxypropan-2-ol (36).- Under an argon atmosphere a stirred solution of (±)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropan-2-ol (29) (0.091g, 0.32mmol), freshly made sodium methoxide (0.021g, 0.039mmol) and anhydrous methanol was boiled under reflux for 18 hours. T.l.c., (diethyl ether : light petroleum ether (b.p. = 40-60 °C); 1 : 1), R_f (29) = 0.31, R_f (28) = 0.43, R_f (36) = 0.17. The reaction mixture was cooled and diluted with water (10ml), the solution was extracted with diethyl ether (3x15ml), dried ($MgSO_4$) and evaporated under reduced pressure. FC (silica 14g, diethyl ether : light petroleum ether (b.p. = 40-60 °C); 1 :1) yielded (±)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy-1,2-epoxypropane (28) (0.048g, 60% yield) as a colourless oil (^1H n.m.r. and t.l.c. identical to

previously synthesised (28)). And also (36) (0.029g, 32% yield) as a colourless oil; δ_H (220 MHz; solvent $CDCl_3$; standard Me_4Si) 0.21 (2H, m, cyclopropane methylene protons), 0.55 (2H, m, cyclopropane methylene protons), 1.05 (1H, m, cyclopropyl methine proton), 2.65 (1H, bm, O*H*), 2.87 (2H, d, *J* 9 Hz, ArCH_2), 3.42 (2H, d, *J* 7 Hz, OCH_2-cyclopropyl protons), 3.45 (3H, s, OCH_3), 3.65 (2H, t, *J* 9Hz, ArCH_2CH_2), 4.03 (2H, m, ArOCH_2), 4.20 (2H, m, CH_2Cl), 4.20 (1H, m, *CH*OH), 6.91 (2H, d, J_{ortho} 9 Hz, Ar-*H*), and 7.22 (2H, d, J_{ortho} 9 Hz, Ar-H); δ_C (100 MHz; solvent $CDCl_3$) 2.82 (C-12), 10.50 (C-11), 35.38 (C-8), 59.13 (C-1''), 68.96 (C-2), 69.02 (C-3), 71.63 (C-10), 73.43 (C-1), 75.45 (C-9), 114.43 (C-5), 129.72 (C-6), 131.57 (C-7), and 156.98 (C-4); m/z (EI) 280 (M^+, 40%), 195 (76), 121 (16), and 107 (100); HRMS, found (m/z); 280.1683, $C_{16}H_{24}O_4$ requires: 280.1673.

(±)-3-[4-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-2-hydroxypropylamine (37).[104] - To a stirred solution of concentrated aqueous ammonia (S.G. = 0.88, 2ml) and methanol (0.5ml), was added (±)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy-1,2-epoxypropane (28) (0.119g, 0.48mmol dissolved in methanol (1ml)) and stirred at room temperature for 15 hours. T.l.c., (dichloromethane : methanol; 5 : 1), R_f (28) = 0.87, R_f (37) = 0.12. The resulting white suspension was filtered and the filtrate evaporated under reduced pressure. FC (silica 14g, CH_2Cl_2 (100%); to CH_2Cl_2 : MeOH; 4 : 1 ; to 2 : 1) gave a white solid (0.105g, 83% yield). (Lit.,[104] 59%, after two recrystallisations), homogeneous by t.l.c. and ^1H n.m.r.; ν ($CDCl_3$) 3 580 w), 3 380 (b OH), 2 920 (s), 2 850 (s), 1 605 (m), 1 580 (w), 1 505 (s), 1 455 (m), 1 290 (m), 1 235 (s), 1 170 (s), 1 085 (s), and 1 040 cm^{-1} (m); δ_H (400 MHz; solvent $CDCl_3$; standard Me_4Si) 0.16 (2H, m, cyclopropane methylene protons), 0.50 (2H, m, cyclopropane methylene protons), 1.02 (1H, m, cyclopropyl methine proton), 2.80 (4H, m, ArCH_2 and CH_2NH_2), 3.24 (2H, d, *J* 7.4 Hz, OCH_2-cyclopropyl proton), 3.40 (3H, b, CHO*H* and NH_2), 3.56

(2H, t, *J* 9Hz, $ArCH_2CH_2$), 3.87 (2H, m, $ArOCH_2$), 3.96 (1H, m, *CH*OH), 6.77 (2H, d, J_{ortho} 8.5 Hz, Ar-*H*), and 7.07 (2H, d, J_{ortho} 8.5 Hz, Ar-H); δ_C (100 MHz; solvent $CDCl_3$) 2.76 (C-12), 10.42 (C-11), 35.26 (C-8), 43.83 (C-1), 69.76 (C-2), 70.07 (C-3), 71.52 (C-10), 75.33 (C-9), 114.31 (C-5), 129.64 (C-6), 131.42 (C-7), and 156.90 (C-4); m/z (EI) 265 (M^+, 9%), 192 (47), 143 (10), 121 (12), 120 (11), 107 (65), 91 (15), 77 (11), 74 (100), and 55 (77); HRMS, found: 265.1670, $C_{15}H_{23}NO_3$ requires: 265.1678. ^1H n.m.r. data equivalent to the Lit.,[104] ^1H n.m.r. data.

(±)-3-[4-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-2-hydroxy-1-(*iso*propyl)propylamine (38). **Method A**.- A solution of (±)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy-1,2-epoxypropane (28) (0.222g, 8.95mmol) in *iso*propylamine (3ml) was stirred under argon for seven days. The solvent was evaporated to yield a white solid (0.208g, 87% yield), homogeneous by ^1H n.m.r. and t.l.c. (T.l.c., (CH_2Cl_2 : MeOH; 5 : 1), R_f (38) = 0.20; (diethyl ether : light petroleum ether (b.p. = 40-69 °C); 2 : 1), R_f (0.0). ^1H n.m.r. identical to (38) produced by **Method B**.

Method B.- Under an argon atmosphere a solution of (±)-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-1-chloropropan-2-ol (29) (0.058g, 0.204mmol) in anhydrous methanol (3ml) and *iso*propanol (87µl, 1.02mmol) was refluxed for 24 hours. T.l.c., (CH_2Cl_2 : MeOH; 5 : 1), R_f (29) = 0.95, R_f (38) = 0.20; (diethyl ether : light petroleum ether (b.p. = 40-60 °C)); 2 : 1, R_f (29) = 0.6, R_f (38) = (0.0). The solvents were evaporated. FC (16g silica ; light petroleum ether (b.p. 40-60 °C) : diethyl ether; 1 : 1, to diethyl ether (100%), to diethyl ether : methanol; 10 : 1, to 1 : 1), gave starting material (29) (0.007g, 12% yield) and a white solid (38) (0.029g, 46% yield) m.p. = 75-77 °C, homogeneous by t.l.c. and ^1H n.m.r.; ν ($CDCl_3$) 3 570 (w), 3 330 (b OH), 2 950 (s), 2 910 (s), 2 850 (s), 1 605 (m), 1 580 (w), 1 505 (s), 1 460

(s), 1 375 (m), 1 330 (m), 1 290 (m), 1 230 (s), 1 170 (s), 1 070 (s), and 1 040 cm^{-1} (m); δ_H (400 MHz; solvent $CDCl_3$; standard Me_4Si) 0.14 (2H, m, cyclopropane methylene protons), 0.46 (2H, m, cyclopropane methylene protons), 1.03 (7H, m, cyclopropyl methine proton and CH_3 X2), 2.25 (2H, bs, OH and NH), 2.62 (1H, m, $NHCHCH_3$), 2.78 (4H, m, $ArCH_2$ and CH_2NH), 3.22 (2H, d, J 6.8 Hz, OCH_2-cyclopropyl protons), 3.55 (2H, t, J 7.4 Hz, $ArCH_2CH_2$), 3.86 (2H, m, $ArOCH_2$), 4.00 (1H, m, $CHOH$), 6.78 (2H, m, J_{ortho} 8.5 Hz, Ar-H), and 7.06 (2H, m, J_{ortho} 8.5 Hz, Ar-H); δ_C (100 MHz; solvent $CDCl_3$) 2.76 (C-12), 10.31 (C-11), 25.17 (C-2'''), 35.17 (C-8), 48.56 (C-1'''), 49.38 (C-1), 68.19 (C-2), 70.63 (C-3), 71.43 (C-10), 75.17 (C-9), 114.20 (C-5), 129.46 (C-6), 131.12 (C-7), and 156.95 (C-4); m/z (EI) 308 ($(M+1)^+$, 18%), 307 (M^+, 47), 263 (5), and 72 (100); HRMS, found (m/z): 308.2205, $C_{18}NO_3H_{30}$ requires: 308.2225; Found (m/z): 307.2176, $C_{18}NO_3H_{29}$ requires: 307.2147.

7.3 EXPERIMENTAL DETAILS FOR CHAPTER THREE.

Methyl β-benzylaminopropionate (46).[121]- To a solution of dry, distilled benzylamine (98.6g, 0.92 mol) in anhydrous methanol (600ml) under a nitrogen atmosphere, was slowly added distilled methyl acrylate (87ml, 0.97mol). The solution was allowed to stand for 24 hours. Methanol was removed by distillation at atmospheric pressure, followed by distillation at reduced pressure to yield (46) (139g, 78% yield) as a clear colourless liquid. (b.p. = 130-135 °C/5mmHg), (Lit.,[121] 92% yield, b.p. = 145-147 °C/7mmHg), homogeneous by ^{1}H n.m.r.; δ_H (200 MHz; solvent $CDCl_3$; standard Me_4Si) 1.63 (1H, bs, NH), 2.53 (2H, t, *J* 7.5 Hz, $NHCH_2CH_2$), 2.90 (2H, t, *J* 7.5 Hz, $NHCH_2CH_2$), 3.68 (3H, s, OCH_3), 3.81 (2H, s, $ArCH_2$), and 7.18-7.49 (5H, m, Ar-H); m/z (EI) 194 ($(M+1)^+$, 57%), 119 (72), 106 (90), and 91 ($C_7H_7^+$, 100).

4-Carbomethoxy-1-benzyl-2,3-dioxopyrrolidine (47).[121]-To a solution of freshly made sodium methoxide (78.5g, 1.45mol) and methyl β-benzylaminopropionate (46) (280, 1.44mol) in dry diethyl ether (1000ml), under nitrogen, was added slowly *via* a dropping funnel a solution of dimethyl oxalate (170g, 1.44mol) in dry diethyl ether (700ml). The white/yellow suspension was stirred for 30 minutes then boiled under reflux for 1.5 hours. The ether was removed under reduced pressure to yield a yellow salt. The solid was dissolved in hot water (2500ml). Then 1M HCl (500ml) was added which caused a white solid to precipitate. The mixture was left to stir overnight. The precipitate was filtered and recrystallised from methanol (6000ml). To give two crops of shiny white crystals (47) (149g, 42% yield) (Lit.,[121] 75% yield), homogeneous by ^{1}H n.m.r.; δ_H (200 MHz; solvent $CDCl_3$; standard Me_4Si) 3.80 (3H, s, OCH_3), 3.85 (2H, s, $ArCH_2$), 4.65 (2H, s, NCH_2C), 7.18-7.40 (5H, m, Ar-H), 9.15 (0.8H, bs, COH), m/z (EI) 247 (M^+, 20%), 215 (64), 187 (23), 130 (53), 106 (100), and 91

($C_7H_7^+$, 100). ^1H n.m.r. and m.s. data equivalent with Lit.,[123] data.

1-Benzylpyrrolidine-2,3-dione (48)[121]. A suspension of 4-carbomethoxy-1-benzyl-2,3-dioxopyrrolidine (47) (30g, 0.121mol) and 20%v/v HCl (600ml) was boiled under reflux for 4 hours, cooled and filtered. The filtrate was extracted with dichloromethane (2x400ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure to give a brown solid. Recrystallised[123] ($CHCl_3$/cyclohexane) to give a whitish powder (48) (10.1g, 44% yield) (Lit.,[121] 69% yield), homogeneous by ^1H n.m.r.; δ_H (200 MHz; solvent $CDCl_3$; standard Me_4Si) 2.73 (2H, t, *J* 6 Hz, NCH_2CH_2), 3.63 (2H, t, *J* 6 Hz, NCH_2CH_2), 4.78 (2H, s, $ArCH_2$), 7.31-7.49 (5H, m, Ar-*H*); m/z (EI) 189 (M^+, 12%), 106 (17), and 91 ($C_7H_7^+$, 100); (CI CH_4) 190 ((M+1)+, 100), 91 ($C_7H_7^+$, 77). ^1H n.m.r. and m.s. data equivalent to Lit.,[123] data.

(3R,S)-1-Benzylpyrrolidine-2-oxo-3-ol (49).- To a cold (-78 °C) stirred solution of lithium-*tert*-butoxyaluminohydride (0.134g, 0.53mmol) in dry THF (3ml) under a nitrogen atmosphere was added slowly over a five minute period 1-benzylpyrrolidine-2,3-dione (48) (0.050g, 0.26mmol dissolved in THF (2ml)). The reaction mixture was left to slowly come to room temperature. The raection mixture was worked-up after 12 hours, t.l.c. (Ethyl acetate : methanol; 10 : 1), R_f (48) = 0.7 and 0.6, R_f (49) = 0.5, 70%w/v ammonium sulphate solution (10ml) was added, diluted with water (30ml) and the solution was extracted with ethyl acetate (3x50ml), dried ($MgSO_4$), filter and evaporated under reduced pressure. FC (3g, ethyl acetate : hexane; 9 : 1) gave a colourless oil (49) (0.03g, 60% yield); δ_H (200 MHz; solvent $CDCl_3$; standard Me_4Si) 1.90-2.05 (1H, m, CH*H*COH), 2.25-2.45 (1H, m, CH*H*COH), 3.10-3.40 (2H, m, NCH_2CH_2), 4.43-4.61 (4H, m, $ArCH_2$, *CH*OH and CHO*H*: this proton is exchangable with D_2O), 7.21-7.45 (5H, m, Ar-*H*); m/z (EI) 191 (M^+, 4%), 173 ($(M-H_2O)^+$, 18), 145, (8), 118, (17), 106 (34), and 91

($C_7H_7^+$, 100). The colourless oil turned brown, the oil was diluted with water, and lyophilised to yield a solid which was recrystallised (diethyl ether/hexane) to give a fluffy white solid (49) (0.009g, 18% yield), homogeneous by t.l.c. and ^1H n.m.r. and identical to data given above.

1-Benzylpyrrolidine-3,3-dimethoxy-2-one (50)[124] - A solution of 1-benzylpyrrolidine-2,3-dione (48) (5.00g, 27mmol), dry trimethyl orthoformate (15ml, 137mmol) and *para*-toluenesulphonic acid, mono hydrate (0.156g, 0.8mmol) in anhydrous methanol (120ml) was boiled under reflux for one hour, (t.l.c. (Ethyl acetate : methanol; 10 : 1), R_f (48) = 0.7 and 0.6, R_f (50) = 0.8). The reaction mixture was cooled, and poured onto ice/H_2O (150ml), extracted with dichloromethane (3x100ml), washed with 1M $NaHCO_3$ (200ml), saturated sodium chloride solution (150ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure to yield a brown liquid. Kugelrohr distillation gave a colourless liquid which solidified on standing to give a white solid (50) (5.21g, 79% yield), homogeneous by t.l.c. and ^1H n.m.r.; δ_H (200 MHz; solvent $CDCl_3$; standard Me_4Si), 2.13 (2H, t, *J* 8.8 Hz, NCH_2CH_2), 3.15 (2H, t, *J* 8.8 Hz, NCH_2CH_2), 3.43 (6H, s, OCH_3 X2), 4.48 (2H, s, $ArCH_2$), 7.15-7.38 (5H, m, Ar-*H*); m/z (EI) 205 ($(M-CH_3OH)^+$, 4%), 144 (4), 106 (5), 91 (36), and 44 (100); m/z (Cl CH_4) 236 ($(M+1)^+$, 3), and 204 (100). ^1H n.m.r. data equivalent to Lit.,[124] data.

4-Carboethoxypyrrolidine-2,3-dione (54).[121] - To a solution of freshly made sodium ethoxide (51g, 0.739mol) in absolute ethanol (500ml), under nitogen was added slowly a solution of β-alanine ethyl ester HCl salt (50.3g, 0.328mol in absolute ethanol (400ml)). The suspension was stirred at room temperature for 90 minutes, then boiled under reflux for 3 hours. The ethanol was removed under reduced pressure to yield a yellow salt. The salt was dissolved in hot water (70 °C, 2500ml), acidified with 20%w/v to

precipitate a solid which was recrystallised from absolute ethanol (600ml), to yield (54) (1st crop: 42g, 75%, 2nd crop: 3g, 6%, total yield 48g, 80%) m.p. = 189-190 °C, (lit.,[121] 185-186 °C, 72% yield); δ_H (200 MHz; solvent $CDCl_3$/5%v/v D6-DMSO; ref. residual $CHCl_3$ δ = 7.24ppm) 1.87 (3H, t, J 7.9 Hz, CH_3CH_2), 2.68 (2H, b, H_2O of hydration ?), 3.46 (2H, s, NCH_2), 3.76 (2H, q, *J* 7.9 Hz, CH_3CH_2), 7.82 (0.9H, bs, N*H*), 9.40 (0.7H, b, CO*H*), m/z (EI) 171 (M^+, 14%), 126 ($(M-CH_3CH_2O)^+$, 44), and 99 ($(M-CO_2CH_2CH_3)^+$, 100)

4-Amino-2-oxo-butanoic acid hydrogenchloride salt (55).[122,124] - A suspension of 4-carboethoxypyrrolidine-2,3-dione (54) (5.49g, 0.032mol) and 20%w/v HCl (140ml) was boiled under reflux for 3.5 hours. T.l.c., (CH_3CN : THF : 1%w/v ammonium hydrogen carbonate solution; 43 : 37 : 20) R_f (54) = 0.72 (u.v. only), R_f (55) = 0.19 (brown to ninhydrin spray). The solvents were removed under reduced pressure to yield a brown solid, which could not be recrystallised. However trituration with absolute ethanol gave slightly off-white lustrous crystals (55) (3.37g, 69% yield), m.p. = 154-155 °C (Lit.,[122] m.p. = 148 °C, 75% yield), homogeneous by t.l.c. and ^1H n.m.r.; ν (nujol mull) 3430 (s), 3 208 (b, COOH), 1 720 (s, C=O), 1 600 (m), 1 580 (m), 1 505 (m), 1235 (m), 1120 (m), 1060 (m), 1040 (m), 950 (m), 905 (m), 840 (m) and 6 95cm^{-1} (m); δ_H (200 MHz; solvent D_2O; standard *H*OD δ = 4.65 ppm), 2.03 (2H, t, *J* 7.5 Hz, NCH_2CH_2), 2.97 (2H, t, *J* 7.5 Hz, NCH_2CH_2), 3.11 (1.8H, s, NH_2DCl ?); δ_H (200 MHz; solvent D6-DMSO; standard $CD_3S(O)CD_2H$ *etc* δ = 2.45 ppm), 2.95 (2H, sex, *J* 7 Hz, NCH_2CH_2), 3.16 (2H, t, *J* 7 Hz, NCH_2CH_2), 8.08 (3H, bs, NH_3Cl). 1H n.m.r. and i.r. data equivalent to Lit.,[122] data.

Methyl-4-amino-2-oxo-butanoate hydrogen chloride salt (58).[128] - To a solution of 4-amino-2-oxo-butanoic acid hydrogenchloride salt (55) (15.4g, 0.1mol) in anhydrous methanol (50ml) was added 2,2-dimethoxypropane

(*ca.* 100ml), this causes precipitation of (55). A further quantity of methanol (60ml) redissolved (55), this was followed by the addition of 2,2-dimethoxypropane (500ml). Stirring for 15 minutes caused complete dissolution of (55). The solution was stirred at room temperature for 4 hours. T.l.c., (methanol : $CHCl_3$: water : HCOOH; 50 : 50 : 15 : 0.5); R_f (55) = 0.4, R_f (58) = 0.75 and 0.80. The solvents were removed under reduced pressure to yield a dark brown solid. Recrystallisation from acetonitrile gave hard yellow crystals (58) (10.1g, 61% yield), m.p. = 112-114 °C (decomposition), homogeneous by ^1H n.m.r. and t.l.c.; δ_H (200 MHz; solvent D6-DMSO; standard $CD_3S(O)CD_2H$ *etc.* δ = 2.45 ppm), 2.96 (2H, sex, *J* 7 Hz, NCH_2CH_2), 3.20 (2H, t, *J* 7 Hz, NCH_2CH_2), 3.76 (3H, s, OCH_3), 8.20 (2.7H, bs, NH_3Cl).

Methyl-4-(benzyloxycarbonyloxy)-4-amino-2-oxobutanoate (60).[129] - To a stirred cold (-12 °C) suspension of methyl-4-amino-2-oxo-butanoate hydrogen chloride salt (58) (0.50g, 3.02mmol) and N-(Benzyloxycarbonyloxy)-succinimide (61) (0.75g, 3.02mmol) in dry dichloromethane (15ml), under an argon atmosphere, was added distilled triethylamine (0.42ml, 3.02mol) and the reaction mixture was left to slowly come to room temperature. T.l.c., chloroform : methanol; 9 : 1, R_f (58) = 0.10 and 0.20, R_f (60), = 0.75, R_f (61) = 0.85. After 14 hours, the reaction mixture was diluted with water (50ml) and extracted with dichloromethane (3x50ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure. FC (silica 30g, hexane : diethyl ether; 1 : 1, to 1 : 4), gave a colourless oil (60) (0.62g, 77% yield), 98% pure by ^1H n.m.r. (2% impurity is the derivitising agent (61)); δ_H (200 MHz; solvent $CDCl_3$; reference residual $CHCl_3$ δ = 7.24ppm), 3.07 (2H, t, *J* 6 Hz, $CH_2C(O)$), 3.47 (2H, q, *J* 6 Hz, $NHCH_2CH_2$), 3.84 (3H, s, OCH_3), 5.04 (2H, s, $ArCH_2$), 5.18 (1H, bs, NH), 7.30 (5H, s, Ar-*H*), m/z (EI) 266 ($M+1)^+$, 4%), 206 (5), 159 (100), and 107 (>100), 91 ($C_7H_7^+$, >100); m/z (Cl CH_4) 266 ($M+1)^+$, 5%), 220 (21), 159 (49), 152 (53), 131 (66), 107 (59), and

91 ($C_7H_7^+$, 100).

(2R,S)-Methyl-4-(benzyloxycarbonyloxy)-4-amino-2-hydroxy butanoate (62).[130] - To a cold (-13 °C) stirred solution of methyl-4-(benzyloxycarbonyloxy)-4-amino-2-oxo butanoate (60) (0.23g, 0.91mmol) in dry THF (10ml) was added sodium borohydride (0.048g, 1.3mmol) and the mixture was stirred for twenty minutes. T.l.c., (chloroform : methanol; 50 : 1), R_f (60) = 0.51, R_f (62) = 0.21. The solution was diluted with water (3ml) stirred at room temperature for 5 minutes then 1M HCl (10ml) was added. The reaction mixture was extracted with diethyl ether (3x25ml), dried ($MgSO_4$) filtered and evaporated under reduced pressure. FC (silica 80g, chloroform (100%), to chloroform : methanol; 99 : 1) yielded a colourless liquid (62) (0.078g, 32% yield), homogeneous by t.l.c. and 1H n.m.r.; δ_H (200 MHz; solvent $CDCl_3$; standard Me_4Si) 1.72-1.90 (1H, m, CH*H*COH), 1.92-2.16 (1H, m, CH*H*COH), 3.13 (1H, d, *J* 5Hz, CHO*H*, exchangable with D_2O shake), 3.47 (2H, sex, *J* 7. Hz, NCH_2CH_2), 3.78 (3H, s, OCH_3), 4.25 (1H, 'quintet', *J* 5 Hz, *CH*OH, in a D_2O shake the 'quintet' collapses to a dd, *J* 5 Hz), 5.11 (3H, b, $ArCH_2$ and N*H*), and 7.32 (5H, s, Ar-*H*); m/z (CI CH_4) 268 $(M+1)^+$, and 250 $(M-H_2O+1)^+$.

(2R,S)-Methyl-4-(benzyloxycarbonyloxy)-4-amino-2-((2'R)-2'-methoxy-2'-trifluoromethylphenylacetoxy)butanoate (63).[103] - To a stirred solution of (2R,S)-methyl-4-(benzyloxycarbonyloxy)-4-amino-2-hydroxy butanoate. (62) (0.010g, 0.04mmol) and (S)-(-)-2-methoxy-2-trifluoromethylphenylacetylchloride (0.020g, 0.08mmol) in carbon tetrachloride (2ml), under an argon atmosphere was added dry pyridine (1ml) and 4-dimethylaminopyridine (cat.). The

suspension was stirred at room temperature for 4 hours, t.l.c., (chloroform : methanol; 50 : 1), R_f (62) = 0.2, R_f (63) = 0.5, diluted with saturated copper sulphate solution (10ml) and extracted with diethyl ether (2x10ml), washed with water (10ml), saturated sodium chloride (10ml), dried ($MgSO_4$) filtered and evaporated under reduced pressure. FC (silca 15g, hexane : diethyl ether; 4 : 1, to 2 : 1) gave a colourless oil (62) (0.015g, 79% yield), homogeneous by t.l.c. and ^1H n.m.r.; δ_H (200 MHz; solvent $CDCl_3$; ref residual proton in $CHCl_3$ δ = 7.24) 2.01-2.24 (2H, m, CH_2CO(MPTA)), 3.11 (1H, m, NHC*H*H, (2R,2R') diastereomer), 3.29 (1H, m, NHCHH, (2S,2R') diastereomer), 3.39 (1.5H, s, C(2')-OCH_3, (2S,2R') diastereomer), 3.47 (1.5H, s, C(2')-OCH_3, (2R,2R') diastereomer), 3.55 (1.5H, s, C(O)OCH_3, (2S,2R') diastereomer), 3.76 (1.5H, s, C(O)OCH_3, (2R,2R') diastereomer), 4.63 (0.4H, b, N*H*, (2R,2R') diastereomer), 4.92 (0.4H, b, 4.25 N*H*, (2S,2R') diastereomer), 5.04 (1H, s, Ar-C*H*H, (2R,2R') diastereomer), 5.05 (1H, s, Ar-C*H*H, (2S,2R') diastereomer), 5.14-5.29 (1H, m, C*H*O(MPTA)), and 7.26-7.71 (10H, m, Ar-*H*).

Baker's yeast reduction of methyl-4-(benzyloxycarbonyloxy)-4-amino-2-oxo butanoate (60).- A suspension of warm distilled water (100ml), baker's yeast (10g, Red Star®, obtained from Woodman's, Madison) and sucrose (10g) was stirred for ten minutes. To the fermenting suspension was added methyl-4-(benzyloxycarbonyloxy)-4-amino-2-oxo butanoate (60) (0.066g, 0.27mmol dissolved in absolute ethanol (1ml)) and stirred at room temperature for 1hour 55 minutes. T.l.c., (diethyl ether : hexane; 10 : 1), R_f (60) = 0.72, R_f (62) = 0.48. The suspension was centrifuged (10,000 r.p.m./10 mins). The supernatant was extracted with diethyl ether (3x120ml), dried ($MgSO_4$) and evaporated under reduced pressure. FC (silica 25g, diethyl ether : hexane; 1 : 1, to 2 : 1, to 4 : 1) gave (S)-(+)-(62) (0.033g, 50% yield, $[\alpha]_D$ = +1.4 °(C 2.04, $CHCl_3$). The %ee was determined by synthesising the (R)-Mosher's ester derivative (63) as described above (making sure the

reaction was complete by t.l.c. before work-up). Ester (63) was purified by PLC (hexane : diethyl ether; 1 : 2). The band at Rf (0.7) was removed to yield (63). The ee = 77% (determined at ^1H n.m.r. (200 MHz)).

Other yeast-mediated reductions of methyl-4-(benzyloxycarbonyloxy)-4-amino-2-oxo butanoate (60).- Pure yeast strains were selected from the laboratory culture collection. The yeasts were stored on YM media slopes at +4 °C. The collection was obtained from the late Professor K. Raper (Department of Bacteriology, University of Wisconsin-Madison). If the original culture collection number and source is known then these are included. Culture collection sources are: AATC = American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, USA., NRRL = Northern Utilization Research and Development Division, U.S. Department of Agriculture, Perioa, Illinois, USA., NCYC = National Collection of Yeast Cultures, Norwich, UK. Yeast reduction general procedure.- All operations (until addition of the substrate) were performed under sterile conditions. Vogels media (10ml) (for recipe see below) was innoculated with a loopful of pure yeast cells selected from the culture collection. The flasks were vigorously shaken on a rotary shaker, in a hot room, at 30 °C. After 24 hours a one in ten dilution into fresh Vogel's media (10ml) was performed. After another period of 24 hours substrate (60) (30mg, dissolved in DMSO (0.3ml), final substrate concentration 3mg/ml) was added. The reactions were monitored periodically t.l.c. (hexane : diethyl ether; 1 : 10) R_f (60) = 0.5, R_f (62) = 0.2. When the reaction was complete the suspension was centrifuged (10,000 r.p.m. / 10 minutes). The supernatant was removed. The yeast cell pellet was resuspended in diethyl ether (10ml) and centrifuged (10,000 r.p.m. / 10 minutes). The supernatant was combined with the aqueous supernatant and extracted with diethyl ether (2x20ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure. PLC

(hexane : diethyl ether; 1 : 10) the band at Rf (0.3) was removed, extracted with ethyl acetate and triturated with carbon tetrachloride to yield (62). The ee was then determined by synthesising the (R)-Mosher's ester (63) as described as above (again making sure the reaction was complete by t.l.c. before work-up). In these cases the product Mosher's ester (63) was purified by PLC (hexane : diethyl ether; 1 : 3) the band at R_f = 0.5 was isolated to yield (63) as a colourless oil. The %ee was then determined at ^1H n.m.r. (200MHz, $CDCl_3$). The results are given in Table 3.1, Section 3.5, Chapter 3. The composition of the Vogel's media is as follows:

Composition of Vogel's medium

Constituent	Quantity /1000ml
Yeast extract	5.0g
Casamino acids	5.0g
Dextrose	40.0g
Na_3citrate.5.5H_2O	3.0g
KH_2PO_4	5.0g
NH_4NO_3	2.0g
$CaCl_2.2H_2O$	0.1g
$MgSO_4.7H_2O$	0.2g
Trace elements	0.1ml

Trace element solution

Constituent	g/100ml
Citric acid.$7H_2O$	5.0
$ZnSO_4.7H_2O$	5.0
$Fe(NH_4)_2(SO_4)_2.6H_2O$	1.0
$CuSO_4.5H_2O$	0.25
H_3BO_4	0.05
$NaMoO_4.2H_2O$	0.05
$MgSO_4.H_2O$	0.05

For routine yeast reductions the medium was made up using the above recipe and diluted to one litre using distilled water. Before sterilising the medium, the pH was adjusted to 5.6 - 5.8.

(3S)-3-Hydroxy-2-pyrrolidone (64) (77%ee).[131,132,133] - To a solution, under an argon atmosphere, of (2S)-methyl-4-(benzyloxycarbonyloxy)-4-amino-2-hydroxy butanoate (62) (0.0168g, 0.068mmol, 77%ee *ex* baker's yeast reduction of methyl-4-(benzyloxycarbonyloxy)-4-amino-2-oxo butanoate (60)) in dry methanol was added 10% pallidium on charcoal (*ca.* 10mg). The mixture was evacuated and then flushed with hydrogen gas.

The procedure was repeated three times. The reaction mixture was then stirred under an atmosphere of hydrogen gas for 90 minutes. T.l.c., (chloroform : methanol; 9 : 1) R_f (62) = 0.80, R_f (methyl 4-amino-2-hydroxybutanoate) = 0.05 (pink to ninhydrin). The reaction was filtered through celite®, and washed through with methanol. The filtrate was concentrated under reduced pressure to a volume of 10ml. The clear solution was cooled to +2 °C and ammonia gas was bubbled in over a period of ten minutes. The reaction was sealed (suba-seal) and transfered to a cold room (+4 °C) for 6 days. T.l.c., (CH_3CN : THF : 1%w/v ammonium carbonate solution; 45 : 35 : 20), R_f (methyl 4-amino-2-hydroxybutanoate) = 0.05, R_f (64) = 0.30. (both pink to ninhydrin) The solvents were evaporated to yield a white solid. The solid was triturated with hot dichloromethane (3x3ml), the solvent was evaporated under reduced pressure to yield a white crystalline solid (64) (0.0029g, 43% overall yield, $([\alpha]_D$ = -72.8 °(c 0.29 CHCl3)) (Lit.,[131] $[\alpha]_D$ = -113 °(c 0.77 CHCl3)) homogeneous by t.l.c. clean, ^1H n.m.r. has an impurity peak at δ = 1.1; δ_H (200 MHz; solvent $CDCl_3$; ref residual proton in $CDCl_3$ δ = 7.24 ppm) 1.70-2.20 (1H, m, CH*H*COH), 2.30-2.65 (1H, m, CH*H*COH), 3.23-3.47 (2H, m, NCH_2CH_2), 4.31-4.45 (1.8H, m, C*H*OH, CHO*H*), 6.48 (0.8H, b, N*H*). The ^1H n.m.r. data is equivalent to the Lit.,[133] data.

7.4 EXPERIMENTAL DETAILS FOR CHAPTER FOUR.

4-(Phenylthio)butan-2-one (72).[149] - Terabutylammonium fluoride trihydrate (Aldrich Chemical Company) (1.58g, 0.005mol) was dissolved in dry THF (90ml), under a nitrogen atmosphere and cooled to 0 °C. To the stirred solution was added dry thiophenol (110g, 1mol), followed by the slow dropwise addition over a period of 25 minutes of dry methyl vinyl ketone (85ml, 1.02mol). T.l.c., (hexane : ethyl acetate; 5 : 1) R_f (thiophenol) = 0.9, R_f (72) = 0.5. After one hour an excess of methyl vinyl ketone (5ml, 0.06mol) was added and the solvent removed under reduced pressure. FC (the compound was divided into two batches, silica 2x500g, CH_2Cl_2 : hexanes; 3 : 1, to 5 : 1) gave a slightly yellow oil (72) (135g, 74% yield), homogeneous by ^1H n.m.r. and t.l.c.; δ_H (200 MHz; solvent $CDCl_3$; standard Me_4Si) 2.14 (3H, s, CH_3), 2.75 (2H, t, *J* 7 Hz, $CH_2C(O)$), 3.13 (2H, t, *J* 7 Hz, $PhSCH_2$), and 7.19-7.37 (5H, m, Ph-*H*); δ_C (100 MHz; solvent $CDCl_3$) 27.28 (C-1), 29.71 (C-3), 42.78 (C-2), 126.02 (C-8), 128.72 (C-7), 129.27 (C-6), 135.54 (C-5), and 174.93 (C-2); m/z (EI) 180 (M^+, 100), 165 (3), 137 (36), 123 (21), 110 (50), 109 (39), and 77 (8); HRMS, found (m/z): 180.0610, $C_{10}H_{12}OS$ requires: 180.0608

(3E)-4-(Phenylthio)butan-3-en-2-one (73).[150] - To a stirred, cold (+4 °C), solution of 4-(phenylthio)butan-2-one (72) (0.50g, 2.8mmol) under an argon atmosphere in the dark, was added N-chlorosuccinimde (recrystallised *ex* benzene) (0.41g, 3.1mmol). The colourless solution turned yellow. The reaction was monitored by ^1H n.m.r. an aliquot of the reaction mixture was removed and the solvent evaporated on an vacumn pump; δ_H (72) (200 MHz; solvent $CDCl_3$; standard Me_4Si) 2.14 (3H, s, CH_3), 2.75 (2H, t, *J* 7 Hz, $CH_2C(O)$), 3.13 (2H, t, *J* 7 Hz, $PhSCH_2$), and 7.19-7.37 (5H, m, Ph-*H*); δ_H (chlorosulphide intermediate (77)) (200 MHz; solvent $CDCl_3$;

standard Me_4Si) 2.13 (3H, s, CH_3), 3.05 (2H, t, J 7 Hz, $CH_2C(O)$), 5.56 (1H, t, J 7 Hz, PhSC*H*Cl), and 7.08-7.52 (5H, m, Ph-*H*). All the starting material was consumed after 4 hours. Dry triethylamine (0.41ml, 2.91mmol) was then added and the reaction was stirred at +4 °C, in the dark for 16 hours. ^1H n.mr. analysis revealed the E : Z (73) ratio was 10 : 1. The reaction mixture was filtered, the filtrate was diluted with diethyl ether (100ml), washed with 1M HCl (100ml), water (100ml) and saturated sodium chloride solution (100ml), dried (Na_2SO_4), filtered and evaporated. FC (silica 60g, hexane : dichloromethane; 2 : 3) gave (3Z)-4-(Phenylthio)butan-3-en-2-one (73) (0.050g, 10% yield), as a slightly yellow oil, t.lc., (hexane : dichloromethane; 2 : 3) R_f = 0.16; δ_H (200 MHz; solvent $CDCl_3$; standard Me_4Si) 2.38 (3H, s, CH_3), 6.38 (1H, d, J 9.4 Hz, C*H*C(O)), 7.25 (1H, d, J 9.4 Hz, PhC*H*), and 7.30-7.55 (5H, m, Ar-*H*). And also (3E)-4-(Phenylthio)butan-3-en-2-one (73) (0.120g, 24% yield), as a slightly yellow oil, t.lc., (hexane : dichloromethane; 2 : 3) R_f = 0.10, homogeneous by t.l.c. and ^1H n.m.r.; δ_H (200 MHz; solvent $CDCl_3$; standard Me_4Si) 2.18 (3H, s, CH_3), 5.95 (1H, d, J 14 Hz, C*H*C(O)), 7.38-7.51 (5H, m, Ar-*H*), and 7.69 (1H, d, J 14 Hz, PhC*H*). A co-worker Mr. Qu-Ming Guo later established that if 1M HCl is ommitted from the work-up, then the E : Z ratio remained at 10 :1. ^1H n.m.r. data equivalent to the Lit.,[150] ^1H n.m.r. data.

(3E)-4-Chlorobut-3-en-2-one (78).[152,153] - Into a well stirred (overhead stirrer), cold (-10 °C) solution, under an nitrogen atmosphere, of anhydrous aluminium chloride (146g, 1.1mol) in dry carbon tetrachloride (300ml), was added slowly, over a 40 minute period, dry acetyl chloride (71ml, 1mol). The suspension was stirred for 5 minutes. Then at 0 °C was bubbled in dry acetylene gas for three hours. The reaction was monitored by ^1H n.m.r. (an aliquot (*ca.* 1ml) was removed, poured onto ice (*ca.* 1g) and a ^1H n.m.r. spectra was recorded of the carbon tetrachloride layer. The

ratio of product (78) to acetic acid was used to monitor the extent of reacion, ((78) δ = 2.68ppm, CH_3 : acetic acid δ = 2.11 ppm, CH_3,). After 5 hours (ratio of (78) : acetic acid; 10 : 1) the suspension was carefully poured onto salt/ice (*ca.* 200g), extracted with diethyl ether (2x300ml), washed 5%w/v sodium hydrogencarbonate solution (300ml), water (300ml), dried ($MgSO_4$), filtered to give a brown liquid. The ether was distilled at room pressure. Carbon tetrachloride was removed by distillation at reduced pressure (100 mmHg). Finally (78) was distilled to give a colourless liquid (78) (23g, 25% yield, b.p. = 58 °C /40mmHg) (Lit.,[153] b.p. = 41 °C /21mmHg, yield = 62%), homogeneous by ^1H n.m.r. The liquid solidified to give a white stable solid when stored in the freezer; δ_H (200 MHz; solvent $CDCl_3$; standard Me_4Si) 2.68 (3H, s, CH_3), 6.92 (1H, d, *J* 13 Hz, *CH*C(O)), and 7.71 (1H, d, *J* 13 Hz, CC*H*).

Yeast reduction of (3E)-4-(phenylthio)butan-3-en-2-one (73).- A screen of nine yeasts were set up for the reducion of (3E)-4-(phenylthio)butan-3-en-2-one (73). The yeasts were selected from the laboratory culture collection (for yeast origins see the experimental details in Section 7.3): *Torulopsis ernobii* ATCC 20,000; *Torulopsis ketyr* 1040; *Candida lipolytica* 5699; *Saccharomyces dobzhanskii* 1974; "Sacch" - *Saccharomyces sp.*; *Candida guilliermondii* ATCC 9058; *Brettanomyces anomalus* 9248, hybred of (*Saccharomyces lactis* 1140) 610x1974 and *Kloeckera corticis* 20109. Yeasts were cultured as described in the experimental details for Section 7.3. One yeast reduction was worked-up. The experimental for this particular reduction is given below; A conical flask containing Vogel's medium (10ml) (see below) was innoculated with a loopful of *Candida lipolytica* 5699 (previously maintained on a slope of YM medium) and shaken at 30 °C for 40 hours. Then 2.5ml of the mixture was transfered to fresh Vogel's medium (25ml) and shaken at 30 °C for 24

hours. (3E)-4-(phenylthio)butan-3-en-2-one (73) (25mg, 1mg/ml) was added. The reaction was monitored by t.l.c., hexanes : ethyl acetate; 2 ; 1), R_f (73) = 0.65, R_f (72) = 0.58, R_f (71) = 0.43, and R_f (70) = 0.35. The reaction was worked-up after 6 days. The suspension was centrifuged (10,000 r.p.m. / 10mins) and the supernatant was removed. The pellet was resuspended in ethyl acetate (25ml) and centrifuged (10,000 r.p.m. / 10mins) and the supernatant removed. The supernatants were combined and extracted with ethyl acetate (2x30ml), dried ($MgSO_4$) filtered and evaporated under reduced pressure. FC (silica 12g, hexane : ethyl acetate; 12 : 1) gave 4-(phenylthio)butan-2-one (72) (7.9mg, 31% yield) and 4-(phenylthio)butan-2-ol (70) (14.2mg, 55% yield). Total recovery = 86%, no other compounds isolated.

(2RS,3E)-4-(Phenylthio)butan-3-en-2-ol (71).- To a stirred cold (0 °C) solution of (3E)-4-(phenylthio)butan-3-en-2-one (73) (0.050g, 0.28mmol) in anhydrous methanol (2ml) was added sodium borohydride (0.021g, 0.56mmol) and stirred for 22 minutes, (t.l.c., hexanes : ethyl acetate; 1 ; 1), R_f (73) = 0.80, R_f (71) = 0.65. The reaction was diluted with water (10ml), extracted with diethyl ether (2x15ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure to yield a colourless liquid (71) (0.043g, 85% yield), homogeneous by t.l.c. and ^1H n.m.r.; δ_H (200 MHz; solvent $CDCl_3$; standard Me_4Si) 1.31 (3H, d, *J* 6.3 Hz, CH_3), 1.64 (1.2H, bs, CHO*H*), 4.39 (1H, quintet, *J* 6.3 Hz, C*H*OH), 5.88 (1H, dd, *J* 14 Hz and J 6.2 Hz, C*H*C(OH)), 6.42 (1H, d, *J* 14 Hz, PhC*H*), and 7.21-7.45 (5H, m, Ar-*H*).

(2RS,3E)-4-(Phenylthio)butan-3-en-2-(*tert*-butyldimethylsilyloxy)butane (73).- [163] The first step is the same as described above. To a stirred cold (0 °C) solution of (3E)-4-(phenylthio)butan-3-en-2-one (73) (0.301g, 1.7mmol) in anhydrous methanol (10ml) was added sodium borohydride

(0.125g, 3.4mmol) and stirred for 30 minutes, (t.l.c., hexanes : ethyl acetate: 1 : 1). R_f (73) = 0.80, R_f (71) = 0.65. The rection mixture was diluted with water (25ml), extracted with diethyl ether (2x75ml) dried ($MgSO_4$), filtered and evaporated under reduced pressure to yield (71) as a colourless liquid (0.32g, 100% yield), t.l.c. identical to authentic (71). It was used directly in the next step. To a solution, under an argon atmosphere, of (2RS,3E)-4-(phenylthio)butan-3-en-2-ol (71) (0.32g, 1.78mmol) in dry THF (5ml) was added *tert*-butyldimethylsilylchloride (0.30g, 1.95mmol) and dry triethylamine (0.27ml, 1.95 mmol). No reaction was observed. 4-Dimethylaminopyridine (cat.) was added and the reaction stirred overnight. No reaction was observed. After 18 hours the reaction mixture was diluted with diethyl ether (20ml), washed with 10% w/v sodium carbonate solution, then extracted with diethyl ether (3x20ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was dissolved in dry dimethyl formamide (3ml). To the solution under argon was added *tert*-butyldimethylsilylchloride (0.30g, 1.95mmol) and imidazole (0.26g, 3.7mmol). T.l.c., (hexanes : diethyl ether; 4 : 1), R_f (71) = 0.20, R_f (74) = 0.85. After 15 hours the reaction was diluted with water (10ml) and extracted with diethylether (3x20ml), dried ($MgSO_4$) filtered and evaporated under reduced pressure. FC (silica 60g, toluene : hexanes; 1 : 7) gave a colourless liquid (73) (0.245g, 47% overall yield), homogeneous by t.l.c. and ^1H n.m.r.; δ_H (200 MHz; solvent $CDCl_3$ ref standard residual proton in $CDCl_3$ δ = 7.24 ppm) 0.08 (6H, s, Si(CH_3) x2), 0.90 (9H, s, *tert*-butylmethyl protons), 1.25 (3H, d, *J* 6.4 Hz, CH_3), 4.39 (1H, m, *CH*OH), 5.95 (1H, dd, *J* 15 Hz and J 5.2 Hz, *CH*C(OH)), 6.34 (1H, d, *J* 15 Hz and *J* 1 Hz, PhC*H*), and 7.18-7.35 (5H, m, Ar-*H*)

(2RS)-4-(Phenylthio)butan-2-ol (70).- To a cold (0 °C) stirred solution 4-(phenylthio)butan-2-one (72) (0.25g, 1.4mmol) in dry THF (25ml) was

added, in three batches over a 12 hour period, sodium borohydride (0.16g, 4mmol). The reaction was stirred at room temperature for 18 hours. T.l.c., (hexane : ethyl acetate; 3 : 1), R_f (72) = 0.65, R_f (70) = 0.40, diluted with water (20ml) and diethyl ether (30ml). Then cautiously 1M HCl (20ml) was added and the mixture was stirred at room temperature for 10 minutes. The aqueous layer was extracted with diethyl ether (3x75ml), dried ($MgSO_4$) filtered and evaporated under reduced pressure. FC (60g silica, hexane : ethyl acetate; 3 : 1) gave a slightly yellow liquid (70) (0.17g, 66% yield) STENCH ! Homogeneous by ^1H n.m.r. and t.l.c.; δ_H (200 MHz; solvent $CDCl_3$; standard Me_4Si) 1.22 (3H, d, J 6.2 Hz, CH_3), 1.59 (1H, bs, OH, exchangable with D_2O), 1.74 (2H, m, CH_2COH), 3.13 (2H, m, $PhSCH_2$), 3.98 (1H, sex, J 6 Hz, $CHOH$), and 7.14-7.38 (5H, m, Ph-H); δ_C (100 MHz; solvent $CDCl_3$) 22.76 (C-1), 29.58 (C-3), 37.62 (C-4), 66.84 (C-2), 125.53 (C-8), 128.55 (C-7), 128.69 (C-6), and 136.13 (C-5); m/z (EI) 182 (M^+, 100), 164 ($(M-H_2O)^+$, 49), 149 (29), 135 (22), 123 (86), 109 (94), and 77 (38); HRMS, found (m/z): 182.0765, $C_{10}H_{14}OS$ requires: 182.0765.

(2R,S)-4-(Phenylthio)-2-acetoxybutane (85).- To a solution of (2R,S)-4-(phenylthio)butan-2-ol (70) (0.020g, 0.11mmol) in dry dichloromethane (2ml) and acetic anhydride (0.10ml, 1.1mmol), was added dry pyridine (1ml) and 4-dimethylaminopyridine (cat.) and stirred at room temperature for 90 minutes. T.l.c., (hexane : ethyl acetate; 2 : 1), R_f (70) = 0.3, R_f (85) = 0.7. The reaction mixture was diluted with diethyl ether (20ml), washed with 1M HCl (20ml), 5%w/v sodium hydrogen carbonate solution (2x20ml), saturated sodium chloride solution (25ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure to yield a colourless liquid (85) (0.014g, 61% yield), homogeneous by ^1H n.m.r. and t.l.c.; δ_H (200 MHz; solvent $CDCl_3$; standard Me_4Si) 1.22 (3H, d, J 5.5 Hz, CH_3), 1.89 (2H, m, CH_2COAc), 2.02 (3H, s, $C(O)CH_3$), 2.89 (2H, m, $PhSCH_2$), 5.00 (1H,

m, C*H*OAc), and 7.11-7.34 (5H, m, Ph-*H*); δ_C (100 MHz; solvent $CDCl_3$) 19.40 (C-2'), 20.73 (C-1), 29.17 (C-3), 35.06 (C-4), 69.22 (C-2), 125.56 (C-8), 128.46 (C-7), 128.79 (C-6), 135.82 (C-5), and 169.87 (C-1'); m/z (EI) 224 (M^+, 67), 164 ((M-[H_2O+acetate])$^+$, 67), 149 (41), 137 (19), 135 (17), 123 (64), 115 (55), 110 (100), 109 (53), and 77 (24); HRMS, found: 224.0872, $C_{10}H_{16}O_2S$ requires: 224.0871.

Yeast reduction of 4-(phenylthio)butan-2-one (72).- Yeasts was selected from the laboratory culture collection (see the experimental details in Section 7.3). The yeasts reductions were carried out as described above for the yeast reduction of (3E)-4-(phenylthio)butan-3-en-2-one (73). The alcohol (70) was isolated by centrifugation, aqueous extraction and FC as described above. The specific rotation of 4-(phenylthio)butan-2-ol (70) was measured.[155] (n.b. $[\alpha]_D$ = -29 ° (c 1 $CHCl_3$) for optically pure (S)-(-)-(70)). The results are given in Scheme 4.12, Scheme 4.15, Tables 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.8, and 4.9. The composition of Vogel's medium is given in the following table:

Composition of Vogel's medium

Constituent	Quantity /1000ml
Yeast extract	5.0g
Casamino acids	5.0g
Dextrose	40.0g
Na_3citrate.5.5H_2O	3.0g
KH_2PO_4	5.0g
NH_4NO_3	2.0g
$CaCl_2.2H_2O$	0.1g
$MgSO_4.7H_2O$	0.2g
Trace elements	0.1ml

Trace element solution

Constituent	g/100ml
Citric acid.7H_2O	5.0
$ZnSO_4.7H_2O$	5.0
$Fe(NH_4)_2(SO_4)_2.6H_2O$	1.0
$CuSO_4.5H_2O$	0.25
H_3BO_4	0.05
$NaMoO_4.2H_2O$	0.05
$MgSO_4.H_2O$	0.05

For routine yeast reductions the medium was made up using the above recipe and diluted to one litre using distilled water. Before sterilising the medium, the pH was adjusted to 5.6 - 5.8.

Determination of the ee of 4-(phenylthio)butan-2-ol (70).- The alcohol was converted into its corresponding acetate (85). The acetate was then dissolved in a CDCl3/0.03% TMS solution.

Tris[3-(heptafluoropropylhydroxymethylene)-d-camphorato] europium (III) derivative (40 mol% equivalent) was added. The ee was determined at ^{1}H n.m.r. (200MHz). The methyl doublet at C-1 was observed as two doublets. In the cases of high optical purity a small quantity of racemic 4-(phenylthio)-2-acetoxybutane (85) was added and the ^{1}H n.mr. spectra re-recorded.

7.5 EXPERIMENTAL DETAILS FOR CHAPTER FIVE.

Cis-3,6-Diacetoxycyclohexene (94).[166] - A solution of pallidium diacetate (0.70g, 3.1mmol), lithium acetate dihydrate (21.5g, 211mmol), lithium chloride (0.52g, 12.3mmol) and *para*-benzoquinone (1.6g, 14.8mmol) in acetic acid (100ml) was stirred for 35 minutes. To the brown solution was added activated manganese dioxide (6.8g, 78.2mmol) and a black suspension formed. Then 1,3-cyclohexadiene (5g, 62.4mmol) in pentane (200ml) was added. The two-phase reaction mixture was stirred at room temperature for 24 hours. The pentane layer was removed. The aqueous layer was diluted with pentane (300ml) and saturated sodium chloride (200ml) and filtered through celite. The layers were separated. The aqueous layer was extracted with pentane (200ml), hexane (2x100ml) and hexane : diethyl ether; 1 : 1 (2x150ml). All the organic layers were combined, washed with saturated sodium chloride (3x60ml), water (2x40ml), 2M NaOH (3x40ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure to give a yellow oil. FC (silica 350g, hexane : diethyl ether; 7 : 1) gave a colourless liquid (96) (6.7g, 54% yield), homogeneous by t.l.c. and ^1H n.m.r. clean. T.l.c., (hexane : diethyl ether; 1 : 6) R_f (96) = 0.85; δ_H (200 MHz; solvent $CDCl_3$ ref standard residual proton in $CDCl_3$ δ = 7.24 ppm) 1.83 (4H, m, CH_2 x2), 2.03 (6H, s, C(O)CH_2 x2), 5.17 (2H, b, CHOAc x2), 5.86 (2H, s, CH=CH); m/z (Cl CH_4) 199 ((M+1)+, 2%), 157 ((M-acetate+1)$^+$, 8), 140 (>100), 139 (M-acetate-H_2O+1)$^+$, >100), 97 ((M-(acetate x 2)-H_2O+1)$^+$, >100), 96 ((M-(acetate x 2)-H_2O+1)$^+$, >100). ^1H n.m.r. data equivalent to Lit.,[166] data.

Cis-2-Cyclohexene-1,4-diol (96).[166] - A solution of *cis*-3,6-diacetoxycyclohexene (1.04g, 5.6mmol) (94), 2M sodium hydroxide solution (6ml, 12mmol) and methanol (24ml) was boiled under reflux for 25 minutes. The solution was cooled and the solvent was reduced under

reduced pressure. T.l.c., (hexane : diethyl ether; 1 : 4), R_f (94) = 0.80, R_f (96) = 0.15. The residue was diluted with water (5ml) and extracted with diethyl ether (6x10ml), dried ($MgSO_4$) filtered and evaporated under reduced pressure to yield a white solid (96) (0.373g, 62% yield), homogeneous by t.l.c., a few minor impurity peaks by ^1H n.m.r.; δ_H (200 MHz; solvent $CDCl_3$ ref standard residual proton in $CDCl_3$ δ = 7.24 ppm) 1.76 (4H, m, CH_2 x2), 2.71 (2H, bs, CHO*H* x2), 4.08 (2H, b, *CH*OH x2), and 5.79 (2H, s, *CH*=*CH*). ^1H n.m.r. data equivalent to Lit.,[112] data.

Cis-(3SR,6RS)-3-Hydroxy-6-acetoxycyclohexene (93).[175] - To a stirred cold (0 °C) solution of *cis*-3,6-diacetoxycyclohexene (94) (2.5g, 12.6mmol) in anhydrous methanol (25ml) was added potassium carbonate (3.49g, 25mmol) and stirred at 0 °C for exactly 15 minutes. T.l.c., (hexane : diethyl ether; 1 : 3), R_f (94) = 0.75, R_f (93) = 0.25. The suspension was diluted with water (50ml) and extracted with ethyl acetate (3x100ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure. FC (silica 106g, hexanes : diethyl ether; 1 : 2) gave a colourless liquid (93) (0.74g, 47% yield), homogeneous by t.l.c. and ^1H n.m.r.; δ_H (200 MHz; solvent $CDCl_3$ ref standard residual proton in $CDCl_3$ δ = 7.24 ppm) 1.65-1.94 (5H, m, CH_2 x2 and CHO*H*), 2.03 (3H, s, C(O)CH_3) 4.14 (1H, b, *CH*OH), 5.15 (1H, m, *CH*OAc), 5.77 (1H, dd, *J* 9 Hz, and *J* 3 Hz, C=*CH*CHOH), and 5.92 (1H, dd, *J* 9 Hz, and *J* 2 Hz *CH*=CHOAc).

Enzymatic screening experiments for the asymmetric hydrolysis *cis*-3,6-diacetoxycyclohexene (94).- Twenty three hydrolytic enzymes were screened. A general procedure is as follows: A suspension of *cis*-3,6-diacetoxycyclohexene (94) (*ca.* 50mg) and enzyme (*ca.* 20mg) in 200mM phosphate buffer pH 7 (2ml) was stirred vigorously at room temperature. The reactions were monitored periodically by t.l.c. ((hexanes : diethyl

ether; 1 : 4), R_f (94) = 0.8, R_f (93) = 0.4, R_f (96) = 0.15). Reactions that appeared to produce predominantly *cis*-3-hydroxy-6-acetoxycyclohexene (93) were worked-up. The reaction mixture was diluted with water (5ml), extracted with diethyl ether (3x15ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure. FC (silica 17g, hexanes : diethyl ether; 1 : 2) gave (93) as a colourless liquid. The specific rotation of (93) was then measured. If the rotation was large ($[\alpha]_D > 40^o$ (c 1 $CHCl_3$)), then a sample of (93) was dissolved in $CDCl_3$ and mixed with (R)-(-)-2,2,2-trifluoro-1-(anthryl)ethanol (2 mol equivalents) and a ^1H n.m.r. (300 MHz) spectra recorded. By integrating the relative acetate (CH_3) resonances of the diastereomeric complexes the ee of (93) was established. The results for the worked-up enzyme reactions are given in Table 5.1, Chapter 5. The enzymes used and their commmercial suppliers are; *Rhizopus niveus* lipase N (Amano), *Aspergillus niger* lipase AP (Amano), *Candida cylindracea* lipase AY (Amano), *Pseudomonas sp.* lipase K-10 (Amano), *Mucor mehei* lipase MAP (Amano), *Geotrichum candidum* lipase GC-4 (Amano), *Hunicola languinosa* lipase R-10 (Amano), *Rhizopus oryzae* lipase FAP (Amano), *Alcaligenes sp.* lipase PL (Meito Sango Ltd), *Candida cylindracea* Lipase OF-360 (Amano), Porcine pancreatic lipase (Sigma), *Chromobacterium viscosum* Lipase CV (United States Biochemical Corporation), Pig liver esterase (Sigma), Electric eel cholinesterase, acetyl (Sigma), *Candida rugosa* Lipase (Amano), *Pseudomonas sp.* Lipase AK (Amano), Lipase L-10 (Amano), *Candida cylindracea* Lipase MY (Meito Sango Ltd), *Rhizopus sp.* Lipase (Serva), *Rhizopus delemar* (Chemical Dynamics Corporation), *Pseudomonas sp.* Lipase P-30 (Amano), α-Chymotrypsin (Sigma), Papain (Sigma), Subtilisin (Sigma).

Irreversible esterifcation of *Cis*-2-Cyclohexene-1,4-diol (96) catalysed from the lipase from *Pseudomonas sp.*- *Cis*-2-Cyclohexene-1,4-diol (96) (30mg,

0.26mmol) was dissolved in a mixture of dry THF (2ml) and distilled isopropenyl acetate (0.12ml, 1.1mmol). Lipase P-30 (Amano) (50mg) was added and stirred at room temperature for 46 hours. T.l.c. diethyl ether, R_f (96) = 0.1, R_f (93) = 0.6, R_f (94) = 0.9. The suspension was filtered and the filtrate evaporated under reduced pressure. FC (silica 12g, hexanes : diethyl ether; 1 : 1) gave a colourless liquid (-)-(3R,6S)-(93) (25.5mg, 62% yield, $[\alpha]_D$ = -32 °(c 2.55 $CHCl_3$), estimated to be 35%ee).

Cis-3,6-Dihexanoyloxycyclohexene (97) and *cis*-(3SR,6RS)-3-hydroxy-6-hexanoyloxycyclohexene (98).- To a stirred cold (0 °C) solution of hexanoic acid (0.11ml, 0.88mmol) in dry dichloromethane (2ml) under an argon atmosphere was added *cis*-2-cyclohexene-1,4-diol (96) (0.050g, 0.44mmol) and 4-dimethylaminopyridine.[108] The suspension was stirred for 15 minutes at room temperature for complete dissolution of (96). The solution was then again cooled to 0 °C and dicyclocarbodiimide (0.18g, 0.88mmol) was added. The reaction was allowed to slowly come to room temperature. T.l.c., (hexanes : diethyl ether; 1 : 1), R_f (96) = 0.05, R_f (98) = 0.25, R_f (97) = 0.40. After 18 hours the reaction mixture was filtered and the filtrate was evaporated under reduced pressure. The solid residue was resuspended in dichloromethane (10ml) and filtered. The filtrate was washed with 1M HCl (10mlx2), saturated sodium hydrogen carbonate solution (10ml), dried ($MgSO_4$) filtered and evaporated under reduced pressure. FC (silica 17g, hexanes : diethyl ether; 20 : 1 to 4 : 1 to 1 : 1) gave two yellow liquids both homogeneous by t.l.c. and ^1H n.m.r.;

cis-(3SR,6RS)-3-hydroxy-6-hexanoyloxycyclohexene (98); δ_H (200 MHz; solvent $CDCl_3$ ref standard residual proton in $CDCl_3$ δ = 7.24 ppm) 0.82 (3H, t, *J* 7 Hz, CH_3), 1.15-1.53 (4H, m, $CH_3CH_2CH_2$), 1.47-1.94 (7H, m, $CH_3CH_2CH_2CH_2$, CH_2CH_2CHOH and CHO*H*), 2.27 (2H, t, *J* 7.7 Hz, $C(O)CH_2$), 4.15 (1H, b, *CH*OH),

5.17 (1H, m, C*H*OAc), 5.75 (1H, dd, *J* 9 Hz, and *J* 3 Hz, C=C*H*CHOH), and 5.93 (1H, dd, *J* 9 Hz, and *J* 2 Hz C*H*=CHOC(O)); *cis*-3,6-dihexanoyloxycyclohexene (97); δ_H (200 MHz; solvent $CDCl_3$ ref standard residual proton in $CDCl_3$ δ = 7.24 ppm) 0.86 (6H, t, *J* 6.7 Hz, CH_3), 1.15-1.35 (8H, m, $CH_3CH_2CH_2$ x2), 1.47-1.70 (4H, m, $CH_3CH_2CH_2CH_2$ x2), 1.74-1.91 (4H, m, CH_2 x2), 2.27 (4H, t, $C(O)CH_2$ x2), 5.17 (2H, m, C*H*OC(O) x2), 5.86 (2H, d, *J* 1 Hz, C*H*=C*H*)

(3RS,4SR)-3-(Ethyl 2'-ethanoate)-4-acetoxycyclohexene (101). [167,170] - A solution of *cis*-(3SR,6RS)-3-hydroxy-6-acetoxycyclohexene (93) (0.40g, 2.56mmol) and *para*-benzoquinone (cat.) in dry triethyl orthoacetate (3.3ml, 18mmol) was heated at 140-150 °C under an argon atmosphere. T.l.c., (hexanes : diethyl ether; 1 : 2) R_f (93) = 0.2, R_f (101) = 0.8. After 14 hours the reaction appeared > 50% complete. Dry triethyl orthoacetate (3.3ml, 18mmol) was added and heated at 140-150 °C. The procedure was repeated after a further 24 hours. After a total reaction time of 62 hours triethyl orthoacetate was removed under reduced pressure (1mmHg). Kugelrohr distillation (oven temperature 85-90 °C/1mmHg) yielded a 9:1 mixture of (101) : (93). FC (silica 30g, hexanes : diethyl ether; 4 : 1) gave a colourless oil (101) (0.29g, 50% yield), homogeneous by t.l.c. and ^1H n.m.r.; δ_H (200 MHz; solvent $CDCl_3$; standard Me_4Si) 1.27 (3H, t, *J* 7 Hz, CH_3CH_2), 1.59-1.95 (2H, m, CH_2C=C), 2.03-2.17 (5H, s and m, C(O)CH_3 and CH_2CHOAc), 2.19 (1H, dd, *J* -15 Hz and *J* 6.7 Hz, C*H*HC(O)OEt), 2.56 (1H, dd, *J* -15 Hz and *J* 6.7 Hz, C*H*HC(O)OEt), 2.91 (1H, m, C*H*C=C), 4.15 (2H, q, *J* 7 Hz, CH_3CH_2), 5.06-5.18 (1H, m, C*H*OAc), 5.46 (1H, dq, *J* 9 Hz and *J* 3 Hz, C*H*=CH), and 5.71 (1H, dq, *J* 9 Hz and *J* 2 Hz, CH=C*H*).

(1RS,6SR)-7-Oxabicyclo[4.3.0]non-2-en-8-one (100). [167,168,170] - Under an argon atmosphere a solution of (3RS,4SR)-3-(Ethyl 2'-ethanoate)-4-acetoxycyclohexene (101) (0.055,

0.24mmol), potassium hydroxide (0.035g, 0.63mmol), methanol (2ml) and water (0.2ml) was stirred for 24 hours. T.l.c., (hexanes : diethyl ether; 1 : 1) R_f (101) = 0.4, R_f (potassium salt intermediate) = 0.0. The solvents were removed under reduced pressure to yield a yellow oil. The oil was acidified with 1M HCl (3ml) and extracted with ethyl acetate (3x7ml), dried ($MgSO_4$) filtered and concentrated under reduced pressure to 3ml. The ethyl acetate solution was diluted with dry THF (1ml). *Para*-toluenesulphonic acid (*ca.* 5mg) was added and the reaction stirred for 12 hours. T.l.c., (hexanes : diethyl ether; 1 : 1) R_f (100) = 0.2. The solution was diluted with saturated sodium hydrogen carbonate solution (7ml) and extracted with ethyl acetate (2x8ml), dried ($MgSO_4$) and evaporated under reduced pressure to yield a slightly yellow oil (100) (0.029g, 87% yield), homogeneous by t.l.c. and ^1H n.m.r.; δ_H (200 MHz; solvent $CDCl_3$; standard Me_4Si) 1.55-1.82 (1H, m, C*H*HCOC(O)), 1.85-2.15 (3H, m, C*H*HCOC(O) and CH_2C=C), 2.24 (1H, dd, *J* -17 Hz and *J* 2 Hz, C*H*HC(O)), 2.73 (1H, dd, *J* -17 Hz and *J* 7 Hz, C*H*HC(O)), 2.85-3.02 (1H, m, C*H*CH2C(O)), 4.64-4.79 (1H, m, C*H*OC(O)), 5.52 (1H, br d, *J* 10 Hz, C*H*=CH), 5.76-5.89 (1H, m, CH=C*H*).

Scale-up of the *Chromobacterium viscosum* Lipase CV-catalysed hydrolysis of *cis*-3,6-diacetoxycyclohexene (94) to produce (+)-(3S,6R)-hydroxy-6-acetoxycyclohexene (93) (*ca* 42%ee).- A suspension of *cis*-3,6-diacetoxycyclohexene (94) (1.02g, 5.2mmol), phosphate buffer 200mM, pH 7 (30ml) and Lipase CV *ex Chromobacterium viscosum* (United States Biochemical Corporation, 17mg, 52,360 units) was stirred at room temperature for 6 hours 35 minutes. The suspension was diluted with water (20ml) and extracted with ethyl acetate (5x50ml), dried ($MgSO_4$) filtered and evaporated under reduced pressure. FC (silica 20g, hexane : diethyl ether; 1 : 2) gave a colourless liquid (+)-(3S,6R)-(93) (0.72g, 90% yield, $[\alpha]_D$ = +36 °(c 0.96 $CHCl_3$), estimated to be 42%ee).

Conversion of (+)-(3S,6R)-3-hydroxy-6-acetoxycyclohexene (93) to (+)-(3S,4R)-3-(ethyl 2'-ethanoate)-4-acetoxycyclohexene (101) and subsequent conversion to (+)-(1S,6R)-7-oxabicyclo[4.3.0]non-2-en-8-one (100).[167,168,170] - The reaction is very similar to the procedures for the racemic compounds as described above. Optically enriched (+)-(3S,6R)-3-hydroxy-6-acetoxycyclohexene (93) (42%ee) was obtained as described above. A solution of *cis*-(+)-(3S,6R)-3-hydroxy-6-acetoxycyclohexene (93) (42%ee) (0.19g, 1.23mmol) and *para*-benzoquinone (cat.) in dry triethyl orthoacetate (3.3ml, 18mmol) was heated at 140-150 °C under an argon atmosphere. T.l.c., (hexanes : diethyl ether; 1 : 2) R_f (93) = 0.2, R_f (101) = 0.8. After 48 hours triethyl orthoacetate was removed at reduced pressure (1mmHg). FC (silica 30g, hexanes : diethyl ether; 9 : 1) gave a colourless oil (+)-(2S,3R)-(101) (0.123g, 44% yield, $[\alpha]_D$ = +24.5 °(c 1.28 $CHCl_3$)), homogeneous by t.l.c. and ^1H n.m.r. and identical to racemic (101). Under an argon atmosphere a solution of (+)-(3S,4R)-3-(ethyl 2'-ethanoate)-4-acetoxycyclohexene (101) (0.050g, 0.22mmol), potassium hydroxide (0.045g, 0.88mmol), methanol (2ml) and water (0.2ml) was stirred for 18 hours. T.l.c., (hexanes : diethyl ether; 1 : 1) R_f (101) = 0.4, R_f (potassium salt intermediate) = 0.0. The solvents were removed under reduced pressure to yield a yellow oil. The oil was acidified with 1M HCl (3ml) and extracted with ethyl acetate (3x7ml), dried ($MgSO_4$) filtered and concentrated under reduced pressure to 3ml. The ethyl acetate solution was diluted with dry THF (1ml) *para*-toluenesulphonic acid (*ca.* 5mg) was added and the reaction stirred for 3 hours. T.l.c., (hexanes : diethyl ether; 1 : 1) R_f (100) = 0.2. The solution was diluted with saturated sodium hydrogen carbonate solution (8ml) and extracted with ethyl acetate (3x10ml), dried ($MgSO_4$) and evaporated under reduced pressure. FC (silica 12g, hexanes : diethyl ether; 1 : 1) gave a colourless oil

(+)-(1S,6R)-7-oxabicyclo[4,3,0]non-2-en-8-one (100) (0.021g, 70% yield, $[\alpha]_D$ = +9.2 ^{o} (c 2.12 MeOH) (Lit.,[168] $[\alpha]_D$ = +28 ^{o} (c 0.6 MeOH), Lit.[167,170] $[\alpha]_D$ = +30 ^{o} (c 1.1 MeOH)), homogeneous by t.l.c. and ^{1}H n.m.r. and identical to racemic (100).

7.5 EXPERIMENTAL DETAILS FOR CHAPTER SIX.

Ethyl 2,3-dibromo-3-phenylpropionate (129) - This compound was prepared by Dr A. Dachs following the literature method of Abbott and Althousen.[193] Ethyl 2,3-dibromo-3-phenylpropionate (129) is a white crystalline solid m.p. = 77.5-78.5 °C (Lit.,[193] m.p. = 74-75 °C); (t.l.c., (light petroleum ether (b.p. 40-60 °C) : ethyl acetate; 1 : 1, R_f (129) = 0.85; δ_H (400 MHz; solvent $CDCl_3$; standard Me_4Si) 1.36 (3H, t, *J* 7.1 Hz, CH_3CH_2), 4.35 (2H, q, *J* 7.1 Hz, CH_3CH_2), 4.88 (1H, d, *J* 11.8 Hz, PhC*H*), 5.39 (1H, d, *J* 11.8 Hz, $CHCO_2Et$), 7.33-7.42 (5H, m, Ph-*H*) ; δ_C (100 MHz; solvent $CDCl_3$) 13.64, 46.76, 50.50, 62.29, 127.77, 128.60, 129.05, 137.33, and 167.40. m/z (FAB -[3-nitrobenzyl alcohol]) 339 ($(M+1)^+$, 7.6%), 337 ($(M+1)^+$, 12.2), 335 ($(M+1)^+$, 6.7), 257 ((M-Br)+, 40), 255 ($(M-Br)^+$, 44).

Ethyl 3-phenyl-1H-aziridine-2-carboxylate (121)[194] - Into dry freshly distilled DMSO (150ml) was bubbled ammonia gas for fifteen minutes. Then ethyl 2,3-dibromo-3-phenylpropionate (129) (30g, 127mmol) was added. The reaction mixture was sealed (with a suba-seal) and left to stand for 4 days. T.l.c., (light petroleum ether (b.p. 40-60 °C) : ethyl acetate; 1 : 1), R_f (129) = 0.85, R_f (*trans*-121) = 0.75, R_f(*cis*-121) = 0.40. The reaction mixture was worked-up (although the reaction was incomplete). The solution was poured onto ice/water (200g), and the mixture was extracted with dichloromethane (3x200ml), washed with water (2x200ml) dried ($MgSO_4$) filtered and evaporated under reduced pressure. FC (silica 200g, light petroleum ether (b.p. 40-60 °C) : diethyl ether; 5 : 1 to 4 : 1 to 2 : 1 to 1 : 1), gave *trans*-(121) (1.42g, 6% yield) as a colourless liquid, homogeneous by t.l.c. and ^1H n.m.r.; δ_H (400 MHz; solvent $CDCl_3$; standard Me_4Si) 1.30 (3H, t, *J* 7.2 Hz, CH_3CH_2), 2.36 (1H, b, N*H*), 2.59 (1H, d, J_{Trans} 2.3 Hz, PhC*H*) when the aziridine proton at δ – 2.36 ppm was irradiated the coupling constant J_{Trans}

= 2.3 Hz), 3.24 (1H, d, J_{Trans} 2.3 Hz, C*H*CO_2Et), 4.20-4.31 (2H, 12 line multiplet, CH_3CH_2), 7.16-7.38 (5H, m, Ph-*H*) ; δ_C (100 MHz; solvent $CDCl_3$) 13.95, 39.28, 40.11, 61.51, 125.96, 127.50, 128.19, 137.71, and 171.50. m/z. (EI) 192 ((M+1)+, 9%), 191 (M+, 7), 146 (11), 131 (14), 117 (100), 105 (51), 91 (33), and 77 (37); HRMS, found (m/z): 191.0951, $C_{11}H_{13}NO_2$ requires: 191.0946.

The second compound to be eluted from the column was *cis*-(121) (1.40g, 6% yield), a white solid m.p. = 51-52 °C (Lit.,[194] m.p. = 63 °C), homogeneous by t.l.c. and ^{1}H n.m.r.; δ_H (400 MHz; solvent $CDCl_3$; standard Me_4Si) 1.30 (3H, t, *J* 7.2 Hz, CH_3CH_2), 1.85 (1H, b, N*H*), 2.99 (1H, d, J_{Cis} 6.4 Hz, PhC*H*) when the aziridine proton at δ = 1.85 ppm was irradiated the coupling constant J_{cis} = 6.4 Hz), 3.47 (1H, d, J_{Cis} 6.4 Hz, C*H*CO_2Et), 3.88-4.00 (2H, 12 line multiplet, CH_3CH_2), 7.21-7.33 (5H, m, Ph-*H*) ; δ_C (100 MHz; solvent $CDCl_3$) 13.73, 36.99, 40.07 (broad 2xC?), 60.83, 127.33, 127.81, 129.75, 134.74, and 168.80. m/z. (EI) 192 ((M+1)+, 3%), 191 (M+, 5), 181 (6), 146 (14), 131 (3), 117 (100), 105 (7), 91 (16), and 77 (7); HRMS, found (m/z): 191.0958, $C_{11}H_{13}NO_2$ requires 191.0946. ^{1}H n.m.r. equivalent in both cases to Lit.,[194] data.

Hydrolytic enzyme screen for the hydrolysis of *cis*- and *trans*-ethyl 3-phenyl-1H-aziridine-2-carboxylate (121).- The procedure for the enzyme catalysed hydrolysis of *cis*-(121) is as follows. To a solution of racemic *trans*-ethyl 3-phenyl-1H-aziridine-2-carboxylate (121) (*ca.* 50mg) in acetonitrile (0.2ml) was added phosphate buffer 100mM, pH 7 (2ml) and enzyme (10-20mg) and the mixture was stirred vigorously at room temperature. The reaction was monitored by t.l.c., (light petroleum ether (b.p. 40-60 °C) : diethyl acetate; 1 : 1), R_f (*cis*-121) = 0.7. No disappearance of (121) was observed in any case. (See Table 6.1, Chapter 6). The same procedure was used for the hydrolysis of *trans*-(121) except that the liquid substrate (121) was not dissolved in acetonitrile.

Ethyl 3-methyl-2,3-epoxybutanoate (130).[195]- This compound was synthesised by Dr A. Dachs following the literature method of Speziale and Frazier.[195] Ethyl 3-methyl-2,3-epoxybutanoate (130) is a colourless liquid; (t.l.c., (light petroleum ether (b.p. 40-60 °C) : ethyl acetate; 5 : 1), R_f (130) = 0.85, homogeneous by t.l.c. and ^1H n.m.r.; δ_H (400 MHz; solvent $CDCl_3$; standard Me_4Si) 1.18 (3H, t, *J* 7.2 Hz, CH_3CH_2), 1.25 (3H, s, CH_3), 1.30 (3H, s, CH_3), 3.20 (1H, s, CH), and 4.20-4.31 (2H, 14 line multiplet, CH_3CH_2); δ_C (100 MHz; solvent $CDCl_3$) 13.84, 17.82, 23.86, 27.70, 59.45, 60.84, and 168.07. m/z. (EI) 192 ((M+1)+, 9%), 191 (M+, 7), 146 (11), 131 (14), 117 (100), 105 (51), 91 (33), and 77 (37).

Titanium diisopropoxide diazide $[Ti(OPr^i)_2(N_3)_2]$.[196] - To a stirred solution of titanium isopropoxide (97%, Aldrich) (48.6g, 171mmol) in pentane (200ml), under an nitrogen atmosphere, was added slowly over a twenty minute period azido trimethylsilane (90+%, Aldrich) (50.2g, 436mmol) and then stirred at room temperature. After three days the yellow suspension was filtered. The yellow solid was washed with pentane and dried under reduced pressure (0.1mmHg) for four hours to yield a fine yellow powder $[Ti(OPr^i)_2(N_3)_2]$ (38g, 90% yield).

Ethyl 3-methyl-3-azido-2-hydroxybutanoate (124).[197] - To a refluxing suspension of $[Ti(OPr^i)_2(N_3)_2]$ (13.2g, 53mmol) in dry benzene (200ml), under an nitrogen atmosphere, was added a solution of ethyl 3-methyl-2,3-epoxybutanoate (130) (6.93g, 48mmol in dry benzene (20ml)) dropwise over a five minute period. The yellow suspension quickly turned to an orange solution. The reaction was boiled under reflux for one hour t.l.c., (light petroleum ether (b.p. 40-60 °C) : ethyl acetate; 5 : 1), R_f (130) = 0.65 , R_f (124) = 0.45 appeared to show *ca* 50% conversion. More $[Ti(OPr^i)_2(N_3)_2]$

(13.2g, 53mmol), was added. T.l.c. after an further 30 minutes boiling under reflux indicated *ca.* 80% completion. Therefore a further quantity [$Ti(OPr^i)_2(N_3)_2$] (9g, 36mmol) was added. After a total of 4 hours boiling under reflux t.l.c. analysis indicated complete conversion. The solvent was evaporated under reduced pressure. The residue was resuspended in diethyl ether (200ml) and stirred with 5% H_2SO_4 (200ml) for 12 hours. The organic later was removed and the aqueous layer was extracted with diethyl ether (2x200ml). The organic layers were combined and dried over ($MgSO_4$), filtered and evaporated under reduced pressure to give (124) as a yellow liquid (9.81g, 108% yield). It was used directly in the next step. A homogeneous (by t.l.c. and ^1H n.m.r.) sample of (124) was provided after the lipase-catalysed hydrolysis of racemic ethyl 3-methyl-3-azido-2-acetoxybutanoate (124), (see below); ν_{max} (neat) 3 356 (br, OH), 2 986 (s), 2 488 (m), 2 061 (s, N_3), and 1 752 (s, C(O)) cm^{-1}; δ_H (400 MHz; solvent $CDCl_3$; standard Me_4Si) 1.30-1.35 (9H, t and s and s, *J* 7.2 Hz, CH_3CH_2 and CH_3 x2), 3.15 (1H, d, *J* 7.2 Hz CHO*H*), 3.96 (1H, d, *J* 7.2 Hz *CH*OH), and 4.20-4.31 (2H, 16 line multiplet, CH_3CH_2); δ_C (100 MHz; solvent $CDCl_3$) 13.98, 22.78, 22.30, 61.04, 62.86, 76.79 and 172.09; m/z. (CI NH_4) 205 ($(M+NH_4)^+$, 22%), 160 (58), 132 (44), 86 (51), 70 (23), and 56 (77).

Ethyl 3-methyl-3-azido-2-acetoxybutanoate (123) - To a cold (0 °C) stirred solution, under an nitrogen atmosphere, of ethyl 3-methyl-3-azido-2-hydroxybutanoate (124) (9.81g) in dry dichloromethane (200ml) and dry pyridine (30ml) was added 4-dimethylaminopyridine (cat.) and acetic anhydride (100ml). T.l.c., (light petroleum ether (b.p. 40-60 °C) : ethyl acetate; 5 : 1), R_f (124) = 0.45 , R_f (123) = 0.65. After 1 hour the solution was washed with 1M HCl (200ml), saturated sodium hydrogen carbonate solution (200ml), saturated sodium chloride solution (100ml), dried ($MgSO_4$) filtered and evaporated under reduced pressure. FC (silica 60g, light

petroleum ether (b.p. 40-60 °C) : ethyl acetate; 19 : 1) gave a colourless liquid (125) (6.61g, 60% yield), homogeneous by t.l.c. and ^{1}H n.m.r.; ν_{max} (neat) 2 941 (s), 2 497 (m), 2 114 (s, N_3), 1 736 (s, C(O)) cm^{-1}; δ_H (220 MHz; solvent $CDCl_3$; standard Me_4Si) 1.31 (3H, t, J 7.3 Hz, CH_3CH_2), 1.38 (3H, s, CN_3CH_3), 1.42 (3H, s, CN_3CH_3), 2.00 (3H, s, $C(O)CH_3$), 4.29 (2H, q, J 7.3 Hz, CH_3CH_2), and 4.49 (1H, s, CHOAc); δ_C (100 MHz; solvent $CDCl_3$) 13.70, 20.07, 22.44, 23.22, 60.55, 61.36, 77.99, 166.97 and 169.68; m/z. (CI NH_4) 247 ($(M+NH_4)^+$, 3%), 230 $(M+1)^+$, 2), 199 (3), 160 (12), 156 (39), and 56 (79).

(2R,S)-Ethyl 3,3-dimethyl-1H-aziridine-2-carboxylate (125) [179] - A solution of ethyl 3-methyl-3-azido-2-hydroxybutanoate (124) (*ex* FC) (0.079g, 0.42mmol) and triphenyl phosphine (0.23g, 0.88mmol) was boiled under reflux, under a nitrogen atmosphere, for 17 hours. (T.l.c., (light petroleum ether (b.p. 40-60 °C) : ethyl acetate; 9 : 1), R_f (123) = 0.25 , R_f (triphenyl phophine) = 0.8, R_f (triphenyl phosphine oxide) = 0.0, R_f (125) = unknown; The product (125) could not be visualised with any t.l.c. spray reagents tested. Therefore, the reaction was monitored by the disappearance of starting material (124)). The solvent was evaporated under reduced pressure. Crude ^{1}H n.m.r. (220 MHz) revealed that the signal attributable to the methine proton of (123) (δ = 3.96 ppm) was not present. However a singlet at δ = 2.45 ppm was present. FC (silica 6g, light petroleum ether (b.p. 40-60 °C) : ethyl acetate; 19 : 1) gave triphenyl phosphine. Then the eluting regime was altered to light petroleum ether (b.p. 40-60 °C) : ethyl acetate; 1 : 1, then light petroleum ether (b.p. 40-60 °C) : ethyl acetate : methanol; 1 : 1 : 0.5. The latter solvent mixture eluted triphenylphoshine oxide (0.066g). The fractions between the PPh_3 and $P(O)Ph_3$-containing fractions were pooled and evaporated under reduced pressure, to yield (125) as a colourless liquid (0.028g, 47% yield), homogeneous by ^{1}H n.m.r.: δ_H (400 MHz; solvent $CDCl_3$; standard Me_4Si) 1.25-1.33 (10H, m, CH_3CH_2,

$CNCH_3$ x2, N*H* this aziridine proton is exchangable with D_2O), 2.43 (1H, b, C*H*), and 4.16-4.28 (2H, 12 line multiplet, CH_3CH_2); δ_C (100 MHz; solvent $CDCl_3$; standard Me_4Si) 14.14, 18.75, 25.88, 39.44, 42.03, 61.14, and 170.92. m/z. (EI) 143 (M+, 2%), 133 (5), 114 (11), 98 (21), 97 (17), 69 (100) and 59 (46).

Ethyl 3-methyl-3-azido-2(R,S)-[(2'R)-2'-methoxy-2'-trifluoromethyl phenylacetoxy]acetoxybutanoate (131).[103] - A solution of (±)-ethyl 3-methyl-3-azido-2-hydroxybutanoate (124) (0.022g, 0.14mmol), dry pyridine (1ml), dichloromethane (2ml), 4-dimethylaminopyridine (cat.) and (S)-(+)-2-Methoxy-2-trifluoromethylphenylacetylchloride (0.073g, 0.27mmol) was stirred at room temperature for 2 hours 30mins. (T.l.c., (light petroleum ether (b.p. 40-60 °C) : ethyl acetate; 9 : 1) R_f (124) = 0.3, R_f (131) = 0.6. The suspension was diluted with diethyl ether (20ml), and washed with saturated copper sulphate (2x20ml), water (10ml), saturated sodium chloride solution (20ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure. FC (silica 6g, light petroleum ether (b.p. 40-60 °C) : ethyl acetate; 19 : 1) gave a colourless liquid (131) (0.042g, 87% yield); δ_H (400 MHz; solvent $CDCl_3$; standard Me_4Si) 1.26-1.38 (9H, m, CH_3CH_2, $CNCH_3$ x2), 3.54 (1.5H, d, *J* 0.6 Hz, OCH_3), 3.65 (1.5H, d, *J* 1.1 Hz, OCH_3), 4.29 (2H, 20 line multiplet, CH_3CH_2), 4.85 (0.5H, s, C*H*O(MPTA)), 4.89 (0.5H, s, C*H*O(MPTA)), 7.40-7.43 (3H, m, Ar*H*), 7.56-7.58 (1H, m, Ar-*H*), and 7.62-7.64 (1H, m, Ar-*H*); δ_C (100 MHz; solvent $CDCl_3$; standard Me_4Si) 13.84 + 13.89, 22.69 + 22.83, 23.14 + 23.24, 55.26 + 55.59, 60.78 + 60.86, 61.95 + 62.03, 79.46 + 79.54, 124.53, 127.31, 127.64, 128.30, 129.66, 131.40, 131.84, 165.98, 166.15, and 166.39

Ethyl 3-methyl-3-azido-2(-)-[(2'R)-2'-methoxy-2'-trifluoromethyl phenylacetoxy]acetoxybutanoate (131).[103] - The ^1H n.m.r. data for diastereomerically pure (131) derived from optically pure (-)-ethyl 3-methyl-3-azido-2-hydroxybutanoate (124) is as follows; δ_H (400

MHz; solvent $CDCl_3$; standard Me_4Si) 1.28 (3H, *J* 7 Hz, CH_3CH_2), 1.36 (3H, s, $CNCH_3$), 1.38 (3H, s, $CNCH_3$), 3.54 (3H, d, *J* 0.6 Hz, OCH_3), 4.29 (2H, 14 line multiplet, CH_3CH_2), 4.89 (1H, s, CHO(MPTA)), 7.40-7.43 (3H, m, Ar*H*), and 7.56-7.58 (2H, m, Ar-*H*).

Enzymatic screen for the enantioselective hydrolysis of ethyl 3-methyl-3-azido-2-acetoxybutanoate (123).- Nine commercial lipases were screened for their ability to hydrolyse the acetate (123). All the lipases used are listed in Table 6.2, Chapter 6. They were all donated by Biocatalysts Ltd (except for porcine pancreatic lipase which was purchased from Sigma Ltd). A general protocol is as follows:

A suspension of ethyl 3-methyl-3-azido-2-acetoxybutanoate (123) (37-50mg), phosphate buffer 100mM, pH 7 (2ml) and enzyme (21-26mg) was stirred vigorously at room temperature. The reactions were monitored periodically by t.l.c., (light petroleum ether (b.p. 40-60 ^{o}C) : ethyl acetate; 9 : 1), R_f (123) = 0.50 , R_f (123) = 0.35. When the reaction was estimated to be 50% complete, it was worked-up. The reaction mixture was diluted with water (10ml) and extracted with dichloromethane (3x10ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure. FC (silica 14g, (light petroleum ether (b.p. 40-60 ^{o}C) : ethyl acetate; 19 : 1) gave colourless liquids (123) and (124), identified by t.l.c. and ^{1}H n.m.r. The E value was determined in a two step procedure. Firstly ethyl 3-methyl-3-azido-2-acetoxybutanoate (123) and (S)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol (1mol equivalents) were dissolved in D6-Benzene and the %ee of (123) determined at ^{1}H n.m.r. (400 MHz). The product ethyl 3-methyl-3-azido-2-hydroxybutanoate (124) was converted to its corresponding Mosher's ester (131) (as described above, making sure the reaction was complete by t.l.c. before work-up). The %ee was then determined at ^{1}H n.m.r. (400 MHz). The results are given in Table 6.2, Chapter 6.

Scale up of the lipase from *Candida cylindracea*-mediated hydrolysis of ethyl 3-methyl-3-azido-2-acetoxybutanoate (123).- A suspension of ethyl 3-methyl-3-azido-2-acetoxybutanoate (123) (2.04g, 8.9mmol), phosphate buffer 100mM, pH 7 (100ml) and lipase *ex Candida cylindracea* (Biocatalysts Ltd) (1.08g) was stirred vigorously at room temperature for 2 hours 50 minutes. The reaction was diluted with water (150ml) and extracted with dichloromethane (5x200ml) (CARE EMULSIONS!), dried ($MgSO_4$) filtered and evaporated under reduced pressure. FC (silica 60g, light petroleum ether (b.p. 40-60 °C) : ethyl acetate; 19 : 1) gave (123) as a colourless liquid (1.4g, 69% yield, 42%ee, $[\alpha]_D$ = +13.6 °(c 0.98, $CHCl_3$)) and also as a colourless liquid (124) (0.5g, 31% yield, >98%ee, $[\alpha]_D$ = -13.5 °(c 1.03, $CHCl_3$)), both identified by t.l.c. and ^1H n.m.r.

Attempted conversion of optically pure (-)-ethyl 3-methyl-3-azido-2-hydroxybutanoate to optically active ethyl 3,3-dimethyl-1H-aziridine-2-carboxylate (125).[179] - A solution of (-) ethyl 3-methyl-3-azido-2-hydroxybutanoate (124) (0.275g, 1.46mmol, >98%ee) and triphenyl phosphine (0.42g, 1.61mmol) was boiled undee reflux for 3 days 8 hours, under an atmosphere of nitrogen. (T.l.c., (light petroleum ether (b.p. 40-60 °C) : ethyl acetate; 9 : 1), R_f (123) = 0.25 , R_f (triphenyl phophine) = 0.8, R_f (triphenyl phosphine oxide) = 0.0, R_f (125) = unknown. The product (125) could not be visualised with any t.l.c. spray reagents tested. Therefore, the reaction was monitored by the disappearance of starting material (124). The solvent was removed under reduced pressure. FC (silica 25g, light petroleum ether (b.p. 40-60 °C) : ethyl acetate; 19 : 1 gave triphenyl phosphine. Then the eluting regime was altered to light petroleum ether (b.p. 40-60 °C) : ethyl acetate; 7 : 1 to 3 : 1, then light petroleum ether (b.p. 40-60 °C) : ethyl acetate : methanol; 1 : 1 : 0.5). This latter solvent mixture

eluted triphenylphoshine oxide. The fractions between the PPh_3 and $P(O)Ph_3$-containing fractions were pooled and evaporated under reduced pressure, to yield a colourless liquid (0.047g, 22% yield, $[\alpha]_D$ = -30.4 °(c 0.75, $CHCl_3$)). However ^1H n.m.r. analysis revealed ethyl 3,3-dimethyl-1H-aziridine-2-carboxylate (125) and another compound (132) in a 2 : 1; (125) : (132) ratio (see Scheme 6.17).

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